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SEX DIFFERENTIATION IN THE PACIFIC SALMON *ONCORHYNCHUS KETA* (WALBAUM)[1]

BY J. G. ROBERTSON[2]

Abstract

The differentiation of the gonad is described in chum salmon embryos and alevins. Contrary to classical findings in teleosts, sex differentiation in the chum salmon proceeds in the male or female direction without an intermediate female phase. From an initially indifferent gonad there is a progressive development of one sex or the other. The organ forms as a fold from the splanchnic mesoderm and, at the time of first appearance, contains primordial germ cells. These enlarge to form the definitive germ cells which, after a series of divisions, form smaller oogonia or spermatogonia. Oogonia are followed by primary and secondary (growing) oocytes, the appearance of which is the criterion of sex distinction. Spermatogonia continue to multiply but do not undergo growth in the alevin. The ovary develops an open endovarial canal and is supported by a prominent mesovarium. The testis remains small and, in the alevin, develops no ducts. It is suspended by a mesorchium.

Introduction

In the majority of teleosts, both male and female gonads have been considered to pass through a female phase in the course of sex differentiation (1, 2, 14). In the Salmonidae, the manner of sex differentiation has been controversial. Protogynous hermaphroditism (transitory female phase) has been described in both Atlantic salmon, *Salmo salar* (4) and rainbow trout, *S. irideus* (10). Padoa (13), however, found that rainbow trout showed bisexual differentiation without an intermediate female stage but felt that a higher rearing temperature (17–20° C. in contrast to 8–13° C.) was responsible for the difference. In a recent paper, Johnston (7) described sex differentiation in male and female bass, *Micropterus salmoides*, without an intermediate intersexual stage. Thus, the manner of sex differentiation in teleosts is by no means clear.

The present paper attempts to clarify the situation by considering the early development of the gonads in the chum salmon, *Oncorhynchus keta* (Walbaum.)

Materials and Methods

Chum salmon were spawned at Cultus lake, B.C., November 18, 1949, and the eggs incubated in the University of British Columbia's fish hatchery. Samples, taken at weekly intervals, were preserved in 10% formalin or Bouin's

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picric acid – formol–acetic acid. The developmental stages are recorded in time (days) and thermal units (the number of degrees above 32° F. per day, cumulated).

The gonads were studied by gross dissection and microscopically in serial cross and sagittal sections (41 specimens). Six additional embryos were examined from material preserved in 1952 in order to provide additional stages. Germ cell development was also checked in a series of preparations made by students in the Department of Zoology. Slides of chum salmon embryos were lent to the author by Ray Jefferies and pink salmon embryos (*O. gorbuscha*) by Diane Sawyer and William Sleath; it is a pleasure to thank them for these slides.

A dioxan paraffin imbedding method was used, sections were cut at 10 μ, stained with Heidenhain's haemotoxylin and light green or eosin, Mallory's polychrome stain, or occasionally Feulgen stain.

Results

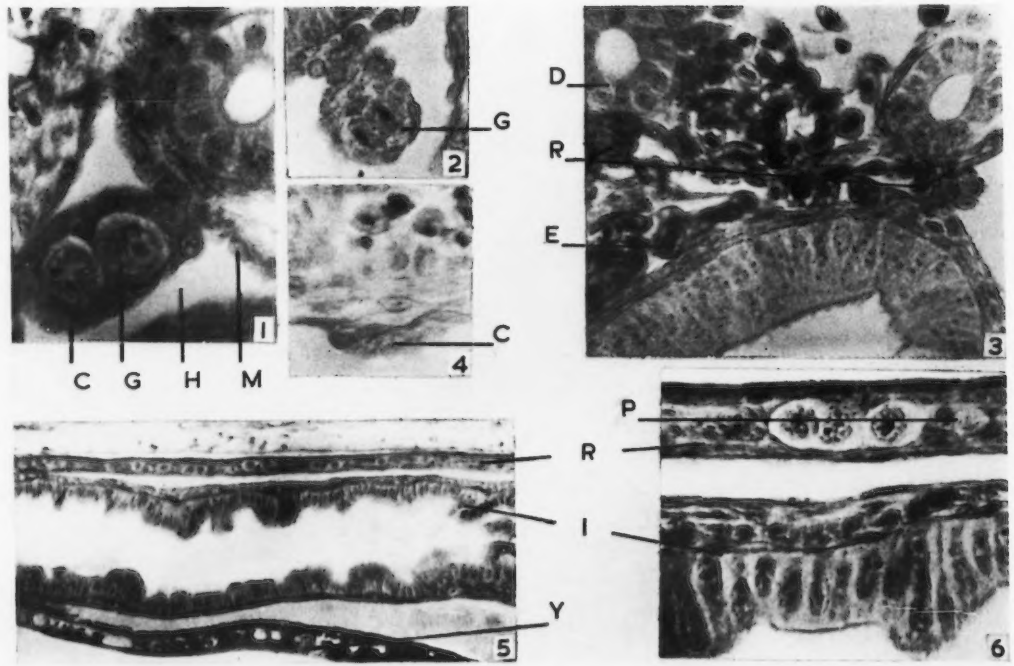
Since different authors have used a variety of terms to describe different stages in the development of egg or sperm, a definition of terms is essential before a clear description of the development can be given. Wilson's (15) terminology will be strictly adhered to in the description that follows. In brief the stages through which the sex cells pass progressively in their differentiation are referred to as stem cell (originating in cleavage and not identified in the present study), primordial germ cell, germ cell, oogonium or spermatogonium, primary oocyte or primary spermatocyte, secondary oocyte or secondary spermatocyte, and finally ovum or sperm. In teleosts, it may be noted, no distinction between primary and secondary oocytes, or primary and secondary spermatocytes seems to have been made.

Differentiation of Germ Cells

97 Days, 920 thermal units (three embryos).

Three embryos were examined for gonad primordia. Two, cut in serial cross section, show paired mesodermal folds attached dorsolaterally to the wall of the splanchnocoel, at the lateral extremity to the mesonephros. These extend from the anterior extremity of the peritoneum to the junction of the paired mesonephric ducts. Here, these folds (gonads) pass insensibly into a connective tissue common to the gut and urogenital sinus (not patent at this time).

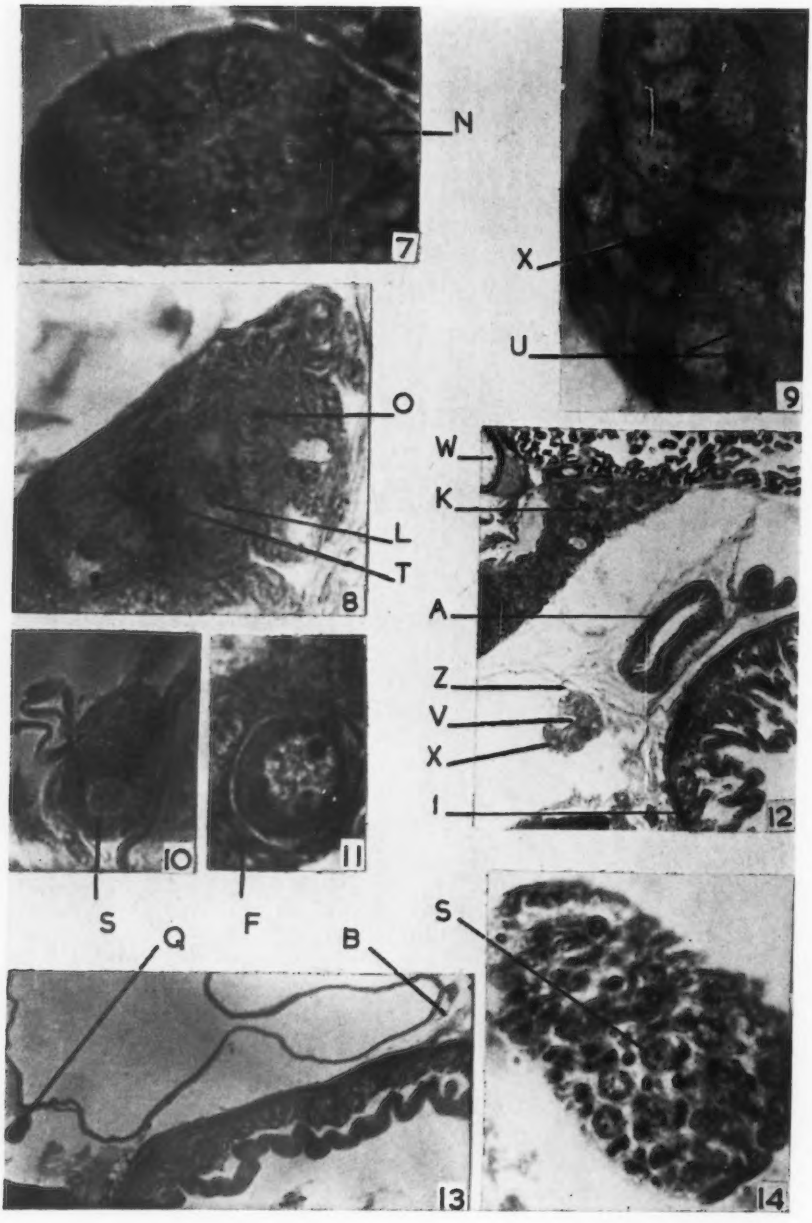
The gonads consist mainly of stroma cells which in section average 4.9 μ (Fig. 3). Among these stroma cells there are groups of larger cells, varying in size from 7.2 to 15.4 μ (Fig. 1). The nucleoplasm of the latter contains a dense chromatin network in sharp contrast to the finely granular cytoplasm. Mitotic figures are occasionally seen in the largest cells (Fig. 2). The smaller cells are clearly germ cell primordia (Fig. 6) from which the large cells by growth become the definitive germ cells. Differentiating germ cells are relatively numerous at the anterior extremity of the developing gonad but decrease



Photomicrographs illustrating the course of sex differentiation.

C, stroma cell; D, mesonephric duct; E, erythrocyte; G, germ cell; H, splanchnocoel; I, intestine; M, splanchnic mesoderm; P, primordial germ cell; R, gonad; Y, yolk.

FIGS. 1–4. Transverse sections through a 97-day embryo, 920 thermal units, at different levels anterior to posterior, showing the origin of gonads. Stroma cells and germ cells are evident. Heidenhain's haemotoxylin. Magn: ×650, ×473, ×571, ×504, in order. FIGS. 5 AND 6. Sagittal section through an embryo just hatched, 1045 thermal units, showing a length of gonad with stem and germ cells; two magnifications. Heidenhain's haemotoxylin. Magn: ×178, ×612, in order.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in number posteriorly. The primordial cells extend farther posteriorly than the large definitive germ cells. Only the stroma cells are found in the posterior sections of the gonad (Fig. 4).

This picture was confirmed in the sagittal sections of a third embryo which shows that the gonads are paired cords of tissue with the germ cells most numerous anteriorly. Primordial and germ cells are supported by stroma cells. The relation of the gonad to the mesonephros and gut are clearly shown in this series.

Period of Germ Cell Multiplication

14 Days prehatching to 42 days posthatching, 980–1560 thermal units (15 embryos).

Primordial cells, initially scattered through most of the gonad, remain characteristically about half the size of the definitive germ cells (Figs. 5, 6). During the next phase of development, germ cells gradually multiply to form large nests (Fig. 7). These groups of germ cells appear first anteriorly and are supported by the stroma cells.

Multiplication of germ cells (in the course of which they become smaller) continues until an almost continuous cord of germ cells, broken only by isolated bands of stroma cells, is evident. The vascularity of the gonad becomes obvious (in midregion) at this time. Caudad few germ cells are present.

Differentiation of the Sexes

55 Days, 1730 thermal units (five alevins)

The five gonads examined fall into two groups. Three of the specimens contain larger gonads with cells (oogonia) which are much smaller (8–9 μ) than the germ cells proper. These oogonia are clearly descended from the small cells of the germ nest. They are occasionally seen in reductional synaptene and diplotene stages and may be referred to as primary oocytes. The other

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Photomicrographs of transverse sections of salmon alevins.

A, air-bladder; B,mesorchium; F, follicle cell; I, intestine; K, kidney; L, synaptene figure; N, germ cell nest; O, oogonia; Q, right testis; S, spermatogonia; T, diplotene figure; U, nucleoli; V, open endovarial canal; W, notochord; X, secondary oocyte; Z, mesovarium.

FIG. 7. 30-day alevin, 1410 thermal units, anteriad, showing gonad containing a nest of germ cells. Mallory's stain. Magn: ×1402. FIG. 8. 55-day alevin, 1730 thermal units, anteriad, showing gonad containing meiotic figures and oogonia. Heidenhain's haemotoxylin and eosin. Magn: ×919. FIG. 9. 62-day alevin, 1825 thermal units, anteriad, showing ovary containing secondary oocytes in different stages of growth. Heidenhain's haemotoxylin and light green. Magn: ×780. FIG. 10. 62-day alevin, 1825 thermal units, anteriad, showing testis with nest of spermatogonia. Mallory's stain. Magn: ×484. FIG. 11. 84-day alevin, 2175 thermal units, showing a secondary oocyte with follicle cells. Heidenhain's haemotoxylin and light green. Magn: ×751. FIG. 12. 84-day alevin, 2175 thermal units, showing paired ovaries with open endovarial canal. Heidenhain's haemotoxylin and light green. Magn: ×69. FIGS. 13 AND 14. 90-day alevin, 2280 thermal units, showing paired testes (Fig. 13) and enlargement of right testis (Fig. 14) to show spermatogonia. Heidenhain's haemotoxylin and eosin. Magn: ×72, ×836, in order.

two specimens contain smaller gonads with germ cell nests. It is suggested on the basis of size that the first group would have developed into females, the second into males.

62 Days, 1825 thermal units (four alevins)

The gonads of the four fish examined fell into three different groups. Two are in an indifferent stage similar to the two specimens described above, one is definitely ovary, and the other testis. The large gonad contains very large cells (oocytes) characteristic of the ovary (Fig. 9). The older cells measure 17.8 μ, a size exceeding the largest germ cell. Moreover, it is clear that the nucleoplasm and not the cytoplasm is the least densely staining part, a feature sharply contrasting with the germ cell (compare Figs. 1, 9). Further, it is evident that they develop from cells (oogonia) intermediate in size between the germ cells and oocytes. The oogonia measure 8.8 μ compared with 13.1–15.4μ for definitive germ cells. At no time are germ cells and oocytes found together. Thus the oogonia arise by multiplication of the germ cells, and the oocytes by differentiation of the oogonia. During this differentiation reduction division occurs, inferring the presence of primary oocytes.

The secondary oocytes are conspicuous by their size and continued growth.

The scanty stroma supporting large cells (spermatogonia), measuring 6–8 μ, characterize the testis (Fig. 10). Germ cells as such are no longer present in these organs. Like the oogonia, the spermatogonia arise in nests resulting from germ cell multiplication and have no clear cell boundaries.

Further Differentiation of Sexes

69–103 Days, 1925–2520 thermal units (5 male, 10 female alevins)

The ovary of the 69-day alevin has a prominent open endovarial canal anteriad and is suspended in the splanchnocoel by a mesovarium (Fig. 12). Oogonia occur and are separated by strands of connective tissue. Stroma cells are comparatively infrequent. At this stage oocytes (secondary oocytes) measure 28 μ, and contain 1–8 or more nucleoli located at the periphery of the nucleoplasm (Figs. 9, 12). Definite follicle cells now surround the oocytes (Fig. 11). The testes, suspended by mesorchia, show little further development (Figs. 13, 14). However, the spermatogonia are no longer in nests but appear as individual cells measuring 7.9 μ in diameter. The nucleoplasm is prominent in these cells (Fig. 14).

In both sexes the gonads increase in length through growth in the posterior portion of the stroma. This portion is now located anterior to the posterior limit of the air bladder, a region marked by the termination of mesonephric tubules.

Discussion

Gonads of chum salmon form as a fold from splanchnic mesoderm walling the coelom. Germinal components are found scattered over the stroma concordant with observations in the Anguillidae and Sparidae (1, 2). In the Amphibia, on the other hand, the presence of germ cells in definite areas is well

established. Amphibian gonads are constituted of medullary (male) and cortical (female) areas. Both contain germ cells. The sexes are gradually emphasized in one or the other of the two areas, depending upon the chemical balance of localized sex determining substances (19). Such topographic areas found in the Amphibia do not occur in chum salmon.

As far as the present investigation is concerned, the origin of primordial germ cells is still in question. As is well known, stem cells can be traced from first cleavage in certain invertebrates (15). In the cyclostomes they are found associated with the yolk entoderm, the primordial cells becoming included in the mesoderm on its differentiation. They are regarded as definitive germ cells when found in the mesenchyme dorsal to the peritoneum (12). In bass (7) primordia are discernible in an excresence at the dorsal lip of the blastopore. Amphibian studies point to a mesodermal origin under the inductive action of the entoderm (11). In the chick primordial cells are said to arise at the primitive streak stage and migrate to the region of the future gonads by way of the blood stream (5). For a review of the germ cell problem in vertebrates see Everett (3). The history of the stem cell did not form a part of the present investigation.

The origin of the definitive germ cell, as described in the present study, does not fall in line with classical observations. In chum salmon germ cells are derived by growth of primordia *located in the differentiating gonad.* Such cells have not been, heretofore, recognized in the gonads of cyclostomes and teleosts. For example, the germ cells in bass are said to lie against, but not in, the gonadal epithelium, and thus the term germinal epithelium has no significance (7). On the basis of the present series of slides there is no evidence contrary to an origin of primordial cells from germinal epithelium as described for higher vertebrates (9). However, in a series of pink salmon embryos examined by the author, cells which are located in an interrenal blastema have every appearance of typical germ cells (not primordial cells). The pink embryos represent earlier stages than here described in the chum salmon series. A careful search for primordial cells in early stages might be profitable.

In chum salmon the definitive germ cells are at least twice the size of their primordia. During the early period of development they decrease in size (by a series of divisions) becoming localized in nests. Thereafter the gonads fall into two groups. The first is marked by an increase in the size of nests owing to multiplication of cells now distinguished as oogonia. In consequence the stroma cells become less conspicuous. At this time the occasional presence of synaptene and diplotene figures suggest that daughters of oogonia (primary oocytes) are present. Comparable figures are described in rainbow trout (13). Gonads showing this development may be tentatively called ovaries. The second group contains nests that show no tendency to increase in size or obscure the stroma cells. The continued presence of such gonads strongly suggests that they will differentiate as testes.

The sexes are clearly separated when the secondary oocytes appear. In contrast with germ cells such oocytes become larger and lack densely reticular

nucleoplasm. With growth of the oocyte, the number of nucleoli increases from one to eight or more. In bass (7) an increase in number of these "numerous rounded bodies" is associated with yolk formation. Certainly yolk material is present at this stage in chum salmon. Oocytes are described in the lamprey *Entosphenus wilderi*, prior to sex differentiation (12). Here the male sex is emphasized by a degeneration of the oocytes and the subsequent multiplication of male germ cells which appear in cysts. No evidence of such a phenomenon could be found in the chum salmon. In chum salmon the smaller, more primitively appearing gonad and nests of spermatogonia distinguish the testes.

In concluding that rainbow trout pass through a female phase before sex differentiations, Mrsic (10) did not study the initial development of the gonad. His first observation, 121 days after hatching, led him to describe gonads with "*mannliche tendenz*" which in chum salmon are testes containing spermatogonia. His second group shows "*weibach tendenz*" corresponding to the appearance of oogonia and secondary oocytes in the present study. In the third group "*erster schritt zur mannlichen tendenz*", he believes that he can distinguish oocytes among male cells. In chum salmon oocytes occur sporadically at first and the attendant germinal elements are oogonia, not male cells. Padoa's (13) studies in rainbow trout clearly show the bisexual pattern of the differentiation.

Why Padoa (13) should have let an otherwise normal state of affairs fall into doubt by attributing his observations to temperature differences, is difficult to understand. He was aware his trout were reared close to lethal temperature, and that such might be expected to upset the sex determining mechanism (16, 17). Therefore, if, as he suggests, trout may be members of sexually undifferentiated or semidifferentiated races (18) equal differentiation of the two sexes could hardly have been expected. The relation of epigenetic factors to sex differentiation in Pacific salmon will be treated by the author in a subsequent paper.

The further development of the ovary shows it to fold dorsolaterally into an open endovarial canal, as noted by Felix (4) in Atlantic salmon and by Padoa (13) in rainbow trout. The testis retains its primitive features through the stages of yolk sac absorption during which time the spermatogonia multiply to cover the stroma, becoming conspicuous cells with prominent reticular nucleoplasm. At later stages it would probably develop a testocoel as observed in Atlantic salmon (8) and as suggested by Padoa (13) for rainbow trout.

In both sexes of chum salmon the germ cells and their products multiply much more rapidly than the stroma cells. At yolk sac absorption the germinal portion was located anteriad to the posterior limit of the air bladder. The stroma extended to the region of the urogenital sinus. At this time the connection between the gonad and the urogenital sinus is not patent. Thus, at the time of yolk-sac absorption, the germinal tissue is largely confined to the anterior half of the gonad, while the posterior half is entirely composed of stroma.

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THE RELATION BETWEEN THERMAL HISTORY AND COLD RESISTANCE IN CERTAIN SPECIES OF RODENTS

By J. Sanford Hart

Abstract

Acclimation of deer mice, white mice, and rats to lower temperatures progressively increased the time to death (resistance time), and lowered the temperature producing death in a given time (resistance temperature). A lower limit to acclimation was reached at 1° to − 10° C. for mice. Sex and body weight affected cold resistance to only a minor extent. Average lethal heat debts were 20 to 24 cal. per gm. for deer mice and white mice. Rats were more resistant than deer mice, and deer mice were more resistant than white mice to low temperatures. No difference in pelage insulation was found in deer mice acclimated to 10° or 30° C.

Introduction

Until recently, little has been published concerning the effects of thermal history on resistance of small mammals to low temperature. Gelineo (11) has shown that the temperature at which rats become hypothermic is markedly influenced by thermal history, and the studies of Blair (2), Sealander (18), and of Sellers *et al.* (19, 21) have shown that acclimation has a definite and measurable effect on the degree of low temperature required to kill deer mice, rats, and rabbits. These workers are all in agreement in that exposure to mild or moderate cold for a period of several weeks or months prolongs the period of time that the animals can withstand more severe degrees of cold, but no quantitative results are available on the magnitude and limits of the increased cold resistance.

In the present study, an attempt has been made to correlate quantitatively with thermal history the resistance to lethal cold of several species when no restriction was placed on the animal's freedom to utilize food resources. In addition, the influence of sex and initial body weight on these results, the heat debt accumulated during exposure, and insulation of the pelage has been investigated.

The term "acclimation" used throughout this study has the meaning employed by Fry and co-workers (4, 9) for poikilotherms, and by Sealander (18) for homoiotherms. It refers to the acquired changes in an animal produced artificially by the alteration of an environmental factor (temperature). The term "acclimatization" is reserved for changes induced by climatic effects.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as N.R.C. No. 2935.

Methods

The effect of thermal history on resistance to low temperatures was determined by acclimating four rodent species to a series of thermal environments and measuring the time to death of groups placed at a series of lethal temperatures. The species used were the forest deer mouse, *Peromyscus maniculatus gracilis* (Le Conte), from the Ottawa region; the northern white-footed deer mouse, *Peromyscus leucopus noveboracensis* (Fischer), from northern New York State (Tumblebrook Farm); white mice, *Mus musculus;* and white rats, *Rattus norvegicus* (Sprague Dawley-Wistar crosses).

All animals used were born and raised in captivity, except for some *P. maniculatus* (Table I, Lot 1) which were trapped and held about one year before being used. The numbers and age of the animals, date of introduction to temperature rooms, periods of acclimation, and lethal test exposures are recorded in Table I. Animals of various ages were distributed at random among the different environments. Each lot of animals, except rats, contained equal numbers of males and females. The rats in Lot 1 were females and in Lot 2 were males (Table I). In addition to the sex difference, rats of Lot 1 differed from those of Lot 2 in their long acclimation period during which tests on tail frostbite were undertaken. *P. maniculatus* acclimated to − 10° C. were exposed first to 10° C. for several weeks (Table I).

TABLE I

AGE AND HISTORY OF ANIMALS

Species	Item		Acclimated to				
			30° C.	22, 20° C.*	10° C.	1° C.	− 10° C.
Peromyscus maniculatus	Acclim. started	Lot 1	25 July, 1950		25 July, 1950		
		Lot 2	19 Dec., 1950		19 Dec., 1950		
		Lot 3		22 Nov., 1951		22 Nov., 1951	24 Jan., 1951
	No. of mice	Lot 1	24		32		
		Lot 2	40		40		
		Lot 3		43		55	46
	Age**	Lot 1	Unknown		Unknown		
		Lot 2	5-10 weeks		5-10 weeks		
		Lot 3		16-29 weeks		23-43 weeks	25-45 weeks
	Acclim. period	Lot 1	8-16 weeks		8-10 weeks		
		Lot 2	12-18 weeks		12-19 weeks		
		Lot 3		7-11 weeks		6 weeks at 10° C.	7 weeks at 10° C.
						7-9 weeks at 1° C.	8 weeks at − 10° C.

* *Peromyscus maniculatus acclimated to 22° C., the others at 20° C.*
** *At start of acclimation.*

TABLE I—*Concluded*

AGE AND HISTORY OF ANIMALS—*Concluded*

Species	Item		Acclimated to				
			30° C.	22, 20° C.*	10° C.	1° C.	− 10° C.
Peromyscus maniculatus—concluded	Lethal test period	Lot 1	8 weeks		8 weeks		
		Lot 2	6 weeks		7 weeks		
		Lot 3		4 weeks		2 weeks	2 days
Peromyscus leucopus	Acclim. started		June 6-8, 1951	June 7, 1951	June 7, 1951		
	No. of mice		55	52	56		
	Age**		5-7 weeks	5-7 weeks	5-7 weeks		
	Acclim. period		8-13 weeks	8-13 weeks	10-13 weeks		
	Lethal test period		5 weeks	5 weeks	3 weeks		
White mice	Acclim. started		12 Sept., 1951	12 Sept., 1951	12 Sept., 1951	12 Sept., 1951	
	No. of mice		40	40	40	40	
	Age**		7-13 weeks	7-13 weeks	7-13 weeks	7-13 weeks	
	Acclim. period		8-12 weeks	8-11 weeks	8-10 weeks	7 weeks	
	Lethal test period		4 weeks	3 weeks	2 weeks	½ week	
White rats	Acclim. started	Lot 1	18 Jan., 1951	7 Jan., 1951	18 Jan., 1951		
		Lot 2			5 Nov., 1951		
	No. of rats	Lot 1	33	35	34		
		Lot 2			17		
	Acclim. period	Lot 1	37-38 weeks	36-39 weeks	33-36 weeks		
		Lot 2			10-13 weeks		
	Lethal test period	Lot 1	1 week	3 weeks	3 weeks		
		Lot 2			3 weeks		

* *Peromyscus maniculatus acclimated to 22° C., the others at 20° C.*
** *At start of acclimation.*

The temperature rooms and cages in which the animals were kept during acclimation were small, and temperatures were controlled within ± 1°C. The air velocity was 11-25 f.p.m. in center of room. The animals were housed singly in open suspended cages provided with a small galvanized iron "nest box" for mice but with no similar protection for rats. "Canary cage" type food cups and water bottles were provided. At first, a liberal amount of excelsior was provided for the mice to discourage their nesting in the food cups, but this bedding was gradually reduced throughout the acclimation period. The position of the cages in each rack was periodically changed so that all animals were exposed to the same environmental conditions. Fluorescent lighting produced an illumination intensity of approximately 60 ft-c. at floor level at a temperature of 20° C. but only 7–25 ft-c. inside the cages. At 0° C. illumination was reduced to 25 and 2–11 ft-c. at floor level and in the cages respectively. The lights were turned on automatically at 6 a.m. and off at 8 p.m.

An effort was made to assess the equivalence of cooling power of environments with the same air temperature by comparing temperature recordings of a copper thimble (1 in. long by $\frac{3}{4}$ in. diam.) heated by a small coil passing a constant amount of current. Changes in surface temperature of the thimble were proportional to the cooling power. The thimble was first calibrated by placing it in a closed brass cylinder 8.3 cm. diam. by 18.6 cm. long and determining thimble surface temperatures for various "still air" temperature values. Equivalent "still air" values for several positions in a 10° C. room and in cages without mice are given in Table II. Actual temperatures were recorded near the thimble. The circulating air in the room lowered the "still air" equivalent by several degrees, but the cage itself afforded considerable protection (about 2° C.), while the cage with insulation raised the equivalent temperature to values higher than the recorded room air (10° C.). Since the mice move about, they are not always thus protected and, because of the wind, they are probably exposed on the average to temperatures somewhat lower than those given by the recorded room air temperatures.

TABLE II

"STILL AIR" EQUIVALENT ENVIRONMENTS AS MEASURED BY HEATED THIMBLE IN VARIOUS POSITIONS

Room temp., ° C.	Still air equivalent, ° C.	Position of thimble
10	5	Center of room
10	6	In cage, floor level
10	7	In cage, 6 ft. above floor
10	11.5	In cage nest box with insulation, floor level
− 20.5	− 18.5	In lethal temp. cage
− 27	− 25	In lethal temp. cage

The rats were fed Master "Fox chow". The diet of the mice, modified from that of Dice (6), is given below:

Ingredient	Per cent by weight	Ingredient	Per cent by weight
Rolled oats	52.0	Canary seed	1.5
Meat scraps	19.0	Millet seed	1.0
Skim milk powder	9.5	Cod liver oil	4.5
Wheat germ	5.0	Sodium chloride	0.5
Whole wheat	5.0	Calcium sulphate	0.5
Sunflower seed	1.5		

After exposure for a minimum period of seven weeks to constant acclimation temperatures (Table I), groups of three males and three females (six males or females for rats) were placed in low temperature environments and the times to death determined. Altogether four or five such groups were removed from each environment (24-30 animals in all) and exposed directly to low temperatures that killed the animals in times varying from 20 to 5000 min. The times to death were determined or estimated and converted to logarithms. The antilog of the mean log time to death is termed "resistance time" of a particular sample. Test temperatures were so chosen that the range of resistance times was similar for animals acclimated to different temperatures. It was thus possible to determine the "resistance temperatures" i.e. lethal temperatures corresponding to given resistance times. In the tests to be described resistance times and temperatures were determined after acclimation at three to five separate temperatures (Table I, Fig. 2).

The terminology used to describe the lethal limits of temperature is similar to that introduced by Fry (9). The lethal effects of temperature are separated into two components, the "incipient lethal temperature" and the "zone of resistance". The incipient lethal temperature is that beyond which 50% of the population can no longer live for an indefinite period of time. At higher temperatures, there is a "zone of tolerance", in which none of the test animals die from the effects of temperature. At lower temperatures, there is a "zone of resistance", in which the animals ultimately succumb to the effects of temperature. "Cold resistance", as measured by resistance time or resistance temperature, refers here only to tests in which there was 100% mortality.

The lethal test cages for mice were 6 × 6 × 6 in. copper boxes with a floor of latex covered wire mesh soldered about one inch from the bottom of the box and with a removable roof made of similar wire mesh. Six cages (one for each mouse) were placed about six inches apart in rooms at the desired temperature. The wind velocity in the center of these rooms ranged from 10-75 f.p.m. and "still air" equivalent environments in the test cages averaged about 2° C. lower than room temperatures (Table II). The mice were placed in the cages in the morning. Food was available at all times in cups small enough to prevent the mice from getting inside. Crushed ice was provided as a water source. Liquid water was supplied once every 24 hr. in tests of long duration.

Temperatures were continuously and automatically recorded by thermocouples placed in the room near the cages, on the walls of the cages, and in the cages near the mice. When the mice were alive, the recorded air temperature in the cages was higher than that of the surrounding air and was used to estimate the point of death within ± 30 min. In short experiments times to death were determined by direct observation.

Lethal test cages for rats were wire mesh cylinders 10 in. diam. by 12 in. high with floors similar to those used in mouse cages. Food, ice, and water were provided and the time to death was either measured directly or estimated from the thermocouple records as described for mice. The arrangement of the cages in the room was similar to that for mouse cages.

Average body temperatures (12) were determined on some of the *P. maniculatus* and white mice at the point of death*. Average initial temperatures were also taken of resting mice exposed to their respective acclimation temperatures. Average lethal heat debts were calculated from these values.

Pelage insulation was determined for *P. maniculatus* acclimated to 30° C. and 10° C. Skins were removed from mice after death at various lethal test temperatures and stored at − 40° C. until ready for test. Before testing, the pelts were extracted with alcohol–ether for two hours. Tests were carried out on a hot cylinder apparatus with copper center cylinder 1.5 cm. diam. × 2.5 cm. long and copper guard cylinders of the same diameter at each end. The cylinders were separated by an air space of 1 mm. and mounted on rubber rings at each end, which in turn were mounted on a glass core. The cylinders were heated independently by nicrome wire on the glass core, one heater coil under each cylinder. The pelt was sewn in place over the center cylinder overlapping the guard cylinders, and by adjustment of the current, the surface temperatures (by thermocouples) of all three cylinders were equalized. The insulation of the pelt was calculated from the current to the center cylinder, and the gradient in temperature from the center cylinder to the surrounding air. Air temperatures were measured by thermocouples on a brass ring 4 in. in diameter surrounding the cylinders. The apparatus was enclosed in a box maintained at approximately 27° C.

Results

Condition of Animals During Acclimation

The physiological condition of the animals differed greatly after acclimation for several weeks to different temperatures. Condition during acclimation was judged by the percentage mortality of the animals and by general appearance of the survivors just before lethal tests were undertaken (Table III). The appearance of the mice at acclimation temperatures of 10° C. and below was inferior to that of mice at higher temperatures and became progressively worse with decrease in temperature. At the lowest temperatures most of the mice

* *Mice were placed in calorimeter when breathing had almost stopped.*

TABLE III

CONDITION OF ANIMALS DURING ACCLIMATION

Species	Item	Acclimated to				
		30° C.	22, 20° C.	10° C.	1° C.	− 10° C.
Peromyscus maniculatus	General appearance*	0	0	1.0	1.3	2.5
	Mortality, %	9	14	17	22	61
Peromyscus leucopus	Mortality, %	7	10	11	—	—
White mice	General appearance*	0	0	1.0	2.4	—
	Mortality, %	10	3	15	60	—
White rats Lot 1	Mortality, %	24	14	32	—	—
Lot 2	Mortality, %	—	—	0	—	—

* *General appearance—figures are averages of grades assigned. Grade 0—no defects, Grade 1—ears partly shriveled, Grade 2—shriveled ears, uneven fur with patches off tail, tail frequently with sores. Grade 3—similar to 2 except more extreme.*

possessed shriveled ears, "patchy" fur, and signs of frostbite. Death of the animals occurred at all temperatures but mortality usually increased with decrease of temperature. At the lowest acclimation temperatures (1° C. for white mice and − 10° C. for *P. maniculatus*) mortality increased to over 50%. The progress of mortality in *P. maniculatus* at − 10°C. is shown in Fig. 1.

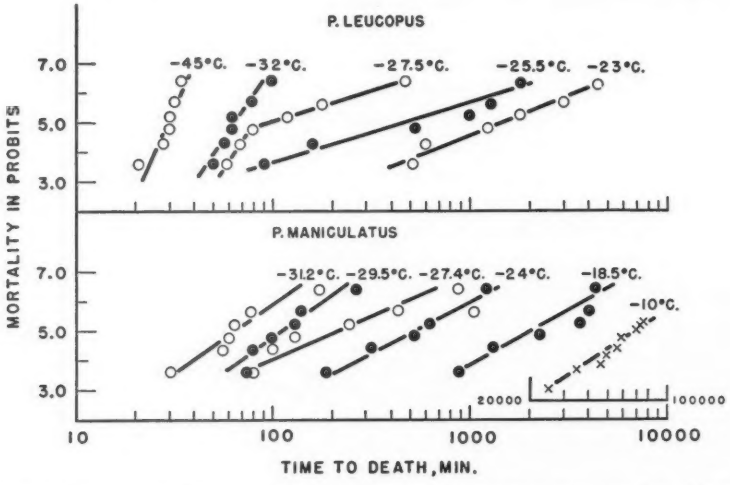

FIG. 1. Time mortality curves for 10° C. acclimated deer mice at various lethal levels of temperature plotted as probits against log time to death. Symbols ○, ●, are used to facilitate observation of curves. Symbol × gives mortality of 10° C. mice placed at − 10° C.

Individual Variation in Cold Resistance

After acclimation (Table I) the mice were subjected to lethal tests. No correction was made for mortality during acclimation. Individual variation in cold resistance differed between species, acclimation temperatures, lots, and exposure temperatures. This variation has been analyzed statistically for *P. maniculatus* acclimated to 30° and 10° C., and is shown graphically (Fig. 1) for the two species of deer mice acclimated to 10° C.

As indicated above, the logarithmic transformation was applied to times to death in order to stabilize intragroup variance, and all tests of significance were made on these transformed data. On this logarithmic scale there was no appreciable difference in variance between exposure temperatures nor between sexes in *P. maniculatus*. However, these mice were tested in two lots (Table I) and the variance of those acclimated at 10° C., unlike those at 30° C., was demonstrably greater in Lot I than in Lot II. Also, the variance of the 10° C. mice was greater than that of the 30° C. mice but this was significant in Lot I only. These results are indicated below:

Lot No.	Variance of log time to death				$F = \frac{\text{var. 10° C. mice}}{\text{var. 30° C. mice}}$
	10° C. mice†	D.f.	30° C. mice	D.f.	
I	.1176	13	.0190	29	6.19**
II	.0359	18	.0190	19	1.89

† $F = \frac{var.\ Lot\ I}{var.\ Lot\ II} = 3.28^*$

* *Significant at 5% level.*
** *Significant at 1% level.*

Individual variation among deer mice acclimated to 10° C. and exposed to various lethal temperatures is shown in Fig. 1. The times to death are plotted against mortality in probits according to the method used by Bliss (3). As indicated above, the variance did not differ between exposure temperatures for *P. maniculatus* and this fact is illustrated by absence of progressive change of slope with change of exposure temperature. In *P. leucopus*, however, the individual variation at − 45° C. and − 32° C. appears distinctly less than at −23° C. and 25.5° C., and the variation at − 27.5° C. appeared greater among the last three mice to die than among the first three. The individual variation of the two species of deer mice at other acclimation temperatures were similar to those illustrated. The variability of white mice and rats also increased progressively with increase in test temperature.

Effect of Exposure Temperature and Acclimation on Cold Resistance

Resistance times increased with increase in exposure temperature and with decrease in acclimation temperature in all species studied and the temperature–time patterns (Fig. 2) were qualitatively similar. Within a given species, the

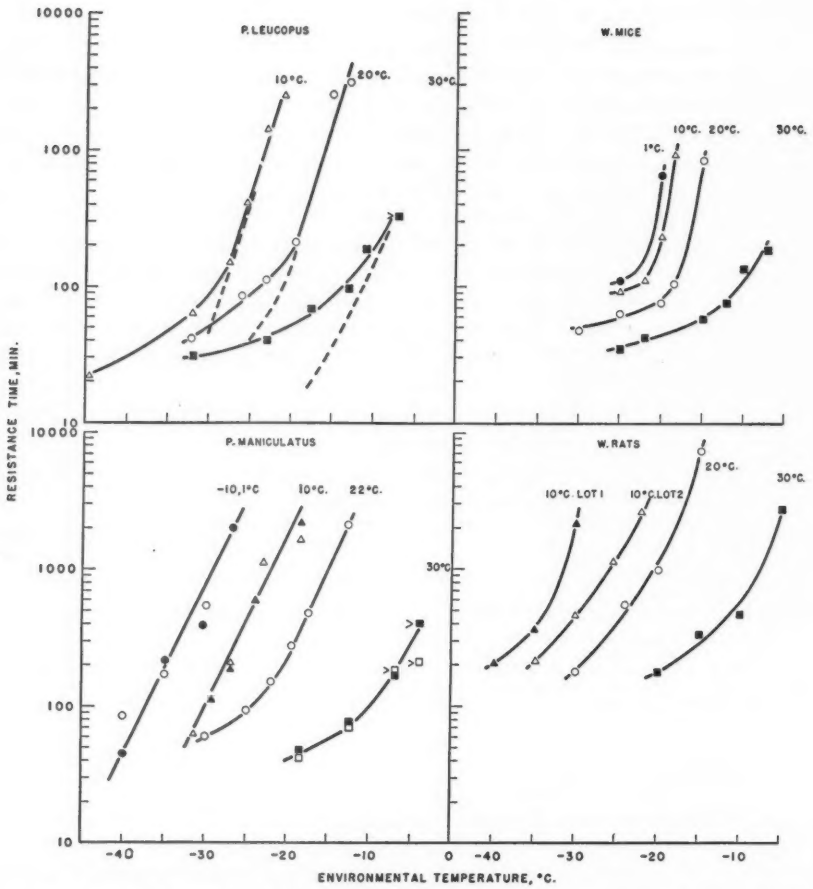

FIG. 2. Resistance times of four rodent species at various temperatures when previously acclimated at temperatures indicated opposite each curve. Dotted lines for *P. leucopus* signify estimated time that metabolism was maintained at normal level. In *P. maniculatus*, △, signifies data for 10° C. acclimated mice (Lot 1); ▲, 10° C. mice (Lot 2); ●, 1° C. mice; ○, − 10° C. and 22° C. mice; □, 30° C. mice (Lot 1); ■, 30° C. mice (Lot 2). Symbol > signifies one to three mice lived for duration of test.

[For rats, *10° C. Lot 1* in the figure should read *10° C. Lot 2* and *10° C. Lot 2* should read *10° C. Lot 1.*]

increase in resistance time with rise in exposure temperature was similar in animals acclimated to different temperatures, with the result that a series of almost parallel curves was found. However, resistance time was not linearly correlated with exposure temperature except possibly in *P. maniculatus* acclimated at temperatures of 10° C. and below. The curves expressing these correlations (fitted by eye, except that for *P. maniculatus* at 10° C. which was fitted by the least square method) were curvilinear at resistance times up to

about 200 min. in mice after which they tended to become straight. (The broken lines for *P. leucopus* derived from data published elsewhere are explained in the discussion.) Data for rats were insufficient to delimit the curvilinearity but there are indications that it was present and possibly extended to longer resistance times than for mice.

The change in resistance temperature for the same change in acclimation temperature was not constant over the temperature range studied (Fig. 2). For a 10° C. change in acclimation temperature, the change of resistance temperature was greatest between 20° and 30° C., and gradually decreased as the acclimation temperature was reduced. These results indicate that a lower limit to acclimation was reached in the neighborhood of 1 to − 10° C. for *P. maniculatus*, and at + 10 to 1° C. for white mice, below which decreased acclimation temperature did not further lower the resistance temperature. These findings are indicated more clearly in Fig. 3, where the resistance temperature for 200 min. resistance time is plotted against acclimation temperature. It will be recalled that the lower limit for acclimation in these species was also characterized by a sudden increase in mortality during acclimation (Table III).

The finding of a lower limit for acclimation at temperature of 1 to − 10° C. in *P. maniculatus* suggests that the incipient lower lethal also falls in this range. Evidence has been obtained that the incipient lethal of 30° C. mice falls between − 2 and − 7° C. and that the incipient lethal of 10° C. mice can be little lower than this. When 30° C. mice were tested at − 4 to − 7°

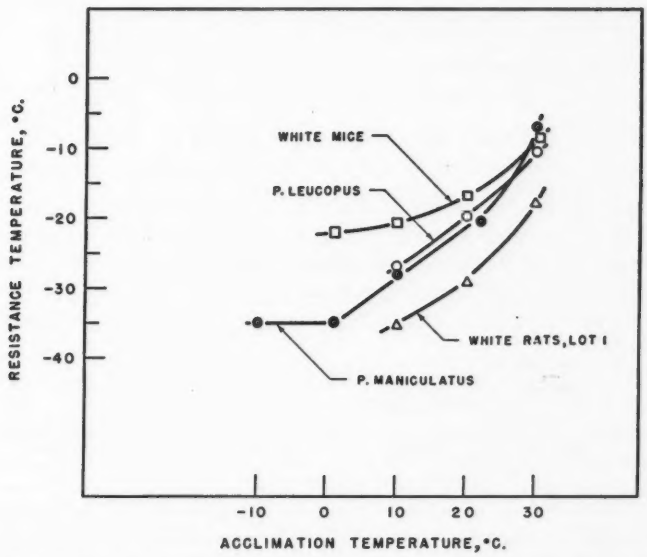

FIG. 3. Resistance temperatures at resistance time of 200 min. in four species of rodents acclimated to various temperatures.

(marked > in Fig. 2, one to three survivors), all deaths occurred in less than 400 min. but the survivors were healthy after three to five days' exposure and there was some regain of weight loss. Two other tests were carried out at 1.5° C. with 30° C. acclimated mice, in each of which only one mouse died. The five survivors in each test were exposed for four days and regained almost all the weight lost initially. When 10° C. mice were exposed to − 10° C. (Lot 3, Table I) 61% died (50% mortality in 74,000 min. and the survivors were not more resistant than 1° C. mice (Fig. 1). Towards the end of their exposure to − 10° C. most mice showed gradual decline in body weight suggesting that this temperature would ultimately cause death.

Effect of Body Weight and Sex on Cold Resistance

Observed differences in survival associated with body weight were interrelated with sex differences since, on the average, males were heavier than females. Except in *P. maniculatus*, average body weight, determined just before each lethal test, decreased progressively with decrease in acclimation temperature (Table IV).

TABLE IV

EFFECT OF ACCLIMATION TEMPERATURE ON BODY WEIGHT

Species	Average weight (gm.) of animals acclimated to				
	30° C.	22, 20° C.	10° C.	1° C.	− 10° C.
Peromyscus maniculatus	23.89	26.27	22.40	24.65	21.23
Peromyscus leucopus	27.90	23.75	22.12	—	—
White mice	29.74	26.75	24.39	22.89	—
Rats (Lot 1)	332	278	258	—	—

In *P. maniculatus* (Lots 1 and 2), body weight had a slight but demonstrable effect on the time of death, the latter tending to increase logarithmically with increasing weight. For every gram increase in weight, time to death increased 17% for 10° C. acclimated mice and 3% for 30° C. mice. The correlation and regression coefficients between weight and time to death were:

	r	b
10° C.	0.49	$.067 \log_{10} \frac{\text{time to death}}{\text{gram}}$
30° C.	0.47	$.014 \log_{10} \frac{\text{time to death}}{\text{gram}}$

However, adjustments of survival time for weight differences according to regression coefficients were minor (Table VII) and did not noticeably reduce the marked differences in time to death at the different exposure temperatures reported above.

TABLE V

BODY WEIGHT IN RELATION TO ORDER OF DEATH

Species	No. of animals	Average weight of animals dying, gm.					
		1st	2nd	3rd	4th	5th	6th
Peromyscus maniculatus	180	22.67	23.98	22.71	25.51	24.97	25.85
Peromyscus leucopus	102	23.60	23.49	24.54	23.66	26.64	25.23
White mice	108	26.58	25.61	25.93	27.25	26.92	26.46
Rats (Lot 1)	72	302	279	293	299	285	292

In Table V average body weight of the animals in all tests is shown relative to order of death. In both species of deer mice, the animals dying earlier in the tests tended to be lighter than those dying later, but no evidence of such a trend was found for white mice or rats.

TABLE VI

EFFECT OF SEX ON RESISTANCE TIME AT VARIOUS ACCLIMATION TEMPERATURES

Species	Sex	Average resistance time* (min.) of animals					Over-all** average
		30° C.	22, 20°C.	10° C.	1° C.	− 10° C.	
Peromyscus maniculatus	♂	81	297	303	208	139	202
	♀	65	281	230	380	339	210
Peromyscus leucopus	♂	73	204	247	—	—	161
	♀	66	195	376	—	—	180
White mice	♂	73	114	370	250	—	145
	♀	83	151	376	297	—	169

** Logs of times to death at all test temperatures were averaged for males and females at each acclimation temperature.*

*** Logs of times to death at all test temperatures regardless of acclimation were averaged for males and females.*

On the average, female mice tended to be slightly more resistant than males (Table VI). Males, however, were more resistant than females at 30° C. and at some intermediate temperatures. In *P. maniculatus* acclimated to 1° C. and below, and in *P. leucopus* acclimated to 10° C. and below, females tended to be more resistant than males. Female white mice were more resistant than males at all acclimation temperatures.

The effect of sex on resistance was not consistent between lots of *P. maniculatus* (Table VII). Sex appeared to affect survival in Lot 2 (10° C. mice) and in Lot 1 (30° C. mice) but sex did not affect survival time in Lot 1

TABLE VII

RESISTANCE TIME OF *Peromyscus maniculatus* AT VARIOUS SUBZERO TEMPERATURES IN RELATION TO BODY WEIGHT AND SEX

Acclim. temp., ° C.	Lot No.	Exposure temp., ° C.	Resistance time, min.					
			Males and females		Males		Females	
			Unadjusted	Adjusted for initial weight	Unadjusted	Adjusted for initial weight	Unadjusted	Adjusted for initial weight
10	1	− 31.2	64	63	—	—	—	—
		− 27.4	209	203	—	—	—	—
		− 23.3	1117	1183	—	—	—	—
		− 18.5	1637	1429	—	—	—	—
	2	− 29.5	116	88	111	90	123	87
		− 27.0	201	223	216	255	183	197
		− 24.0	594	798	1059	1262	333	505
		− 18.5	2188	2218	1589	1393	3972	3532
30	1	− 18.2	44	50	50	53	44	48
		− 12.4	67	68	72	72	63	63
		− 7.2	> 188	> 177	> 356	> 328	123	118
		− 4.0	> 210	> 200	> 342	> 332	> 164	> 157
	2	− 18.5	48	46	—	—	—	—
		− 12.5	73	73	—	—	—	—
		− 7.0	183	207	—	—	—	—
		− 4.0	> 429	> 392	—	—	—	—

NOTE: *In 30° C. mean log survival times preceded by the symbol > are minimum values because one or more animals survived the test period.*

(10° C. mice) or in Lot 2 (30° C. mice). The effect of sex was also not consistent at different exposure temperatures for 10° C. mice, the female resistance time exceeding males at − 29.5 and − 18.5° C., while at − 24.0° C. the opposite was indicated. Some of these differences may be a reflection of differences in origin and age of the mice (old wild mice in Lot 1, domesticated in Lot 2).

Effect of Additional Uncontrolled Factors on Cold Resistance

P. maniculatus acclimated to 10° and to 30° C. were tested in two lots at different seasons of the year (Table I). The mice of the first lot were considerably older than the second, and were not born in captivity. In spite of these differences in history, resistance times were similar (Fig. 2) but differences in variance were found (described in section on individual variation).

In rats acclimated to 10° C., the resistance to cold was greater for animals of the second lot than for those of the first. Therefore, factors in addition to acclimation were effective in changing the cold resistance of these animals. The major differences in history of these animals were those of sex males in Lot 1, females in Lot 2), period of acclimation (Table I), and use of Lot 1 for tail frostbite tests during the acclimation period.

Species Comparisons

Species differences were observed in individual variability, in resistance times, and in resistance temperature. There was a decrease of individual variability of times to death with increase in exposure temperature in *P. leucopus* but not in *P. maniculatus* (Fig. 1). The increase in resistance time for the same increase in exposure temperature was more uniform in *P. maniculatus* than in *P. leucopus* (Fig. 2). Acclimation effected similar changes in cold resistance in both species, but when acclimated to 30° C. *P. leucopus* appeared to resist lower exposure temperatures than *P. maniculatus* (Fig. 3).

White mice were less resistant to low temperatures than either species of deer mouse at acclimation temperatures of 20° C. and below (Fig. 3). The differences became greater as the acclimation temperature decreased. The 200 min. resistance temperature of 1° C. mice was − 22° C. for white mice and − 35° C. for *P. maniculatus*. The increase in resistance temperature for the same decrease in acclimation temperature (acclimation response) was much less in white mice than in deer mice but species differences in resistance temperature of 30° C. acclimated mice were small. At resistance times over 200 min. (Fig. 2), white mice showed greater increases in resistance time than *P. maniculatus* for the same increase in temperature. At resistance times less than 200 min. there was a marked decrease in response to lowering of exposure temperature (change in slope), which was similar to that noted in *P. leucopus*.

White rats were more resistant to cold than either deer mice or white mice, and the acclimation response was similar to that of *P. maniculatus*. Differences in the 200 min. resistant temperature (Fig. 3) between rats and white mice were least at 30° C. and became progressively greater with decrease in

acclimation temperature. Rats showed a decrease in acclimation response with decrease in acclimation temperature similar to that noted for other species, but the acclimation temperatures tested were not low enough to fix the lower acclimation limit.

Body Temperature and Heat Debt

Although deer mice usually maintained normal body temperatures during acclimation (Table VIII), four lethargic mice in Lot 1 at 10° C. were observed. They did not attempt to avoid capture or to escape. The average body temperature of one of these semitorpid mice was 27.1° C.

TABLE VIII

AVERAGE BODY TEMPERATURE AND LETHAL HEAT DEBT

Species	Acclim. temp., ° C.	Initial, average		Lethal, average		Heat debt, average
		Body weight, gm.	Body temp. ° C.††	Body weight, gm.	Body temp. ° C.††	Cal./gm.
Peromyscus maniculatus	10	21.9	36.3 ± 0.4 (9)	19.3	6.7 ± 1.1 (18)	24.6
	30	22.4	37.2 ± 1.0 (8)	23.3	11.6± 0.3(11)**	21.2
White mice	1	—	36.0†	19.3	10.0 ± 1.0 (5)	21.6
	10	—	36.0†	24.4	9.2 ± 0.6 (12)	22.2
	20	—	36.0†	24.1	9.7 ± 0.6 (14)	21.8
	30	—	36.0†	27.9	12.4 ± 0.6 (14)*	19.6

NOTE: *Figures in brackets indicate numbers of animals tested.*

† *Average for white mice taken from literature (12).*
†† *Determined calorimetrically (12).*
* *Difference between 30° C. mice and the other three groups significant at 5% level.*
** *Difference between 10° C. mice and 30° C. mice significant at 1% level.*

Heat debts have been calculated for white mice and *P. maniculatus* directly from the difference between average values for average body temperature (12) of resting nontorpid mice at the end of their period of acclimation (initial) and average values of average (lethal) body temperature at point of death (Table VIII). Initial average body temperatures were slightly higher in deer mice acclimated to 30° C. than in those acclimated to 10° C., but the difference was not significant. Lethal body temperatures were significantly higher in 30° C. deer mice and in 30° C. white mice than those found in the same species acclimated to lower temperatures.

Heat debts incurred during lethal exposure were around 500 cal. per mouse or roughly 21 cal. per gm. There was some indication that heat debts varied with acclimation temperatures; the 10° C. deer mice sustaining a greater heat debt than 30° C. deer mice. Little difference was found between the lethal heat debts of white mice and deer mice. No correlation was found between heat debt and time to death.

Pelage Insulation

Insulation of the fur was measured on 14 *P. maniculatus* (seven males and seven females) acclimated to 10° C. and a similar group acclimated to 30° C. no significant differences were found. The insulation of 10° C. mice averaged .156 ± .009 ° C./cal./m./hr. and that of 30° C. mice averaged .146 ± .006. When expressed in clo units (10) deer mouse pelage averaged approximately 0.8 clo. The fur of most of these mice was very thin and clumping of the hairs from oily deposition was frequently noted.

Discussion

Under natural conditions the organism is continually adjusting to changed environmental conditions, but the adjustment is seldom complete because environmental conditions are changing too rapidly. Under artificial conditions it is possible to determine the influence of a restricted number of environmental factors upon the organism when these factors are imposed for a length of time sufficient for physiological equilibrium to be established. Several studies (8, 11, 14) have shown that acclimation periods of five weeks or longer are required for establishment of equilibrium with the environment. Under these conditions, the present study, and those of previous workers (2, 18, 19, 21), indicate that lethal temperatures provide a precise means of expressing acclimation in quantitative terms.

The species under investigation here were acclimated to three or more temperatures and each acclimation group was tested at a series of lethal temperatures. From these data it was possible to determine to what extent acclimation changed resistance temperature, and it was found that the acclimation response (increase in resistance temperature for the same decrease in acclimation temperature within the species) was not uniform. It was definitely greater at higher acclimation temperatures (exceeding 1° C. per 1° C. change in acclimation temperature) and decreased progressively with decrease in acclimation temperature until a lower limit to acclimation was reached at approximately 0 to − 10° C. for mice. The lower limit was also characterized by marked deterioration in general appearance and by increased mortality during acclimation.

Whether mortality during acclimation influenced the lethal temperature results is not known. If mortality was caused by uncontrolled factors acting at random, no effect on the results would be anticipated. Another possibility is that mortality resulted in survival of more resistant animals. These survivors may, on the other hand, be less resistant at the time of testing than they were earlier in the acclimation period, i.e. approaching the "stage of exhaustion" (22). No attempt has been made to correct for the effect of acclimation mortality on cold resistance.

The lethal effects of low temperatures have been divided into two components (*a*) the zone of resistance in which 100% of the exposed individuals are killed and (*b*) the incipient lethal level in which only 50% of the exposed animals are eventually killed by temperature. Although acclimation produces marked changes on lethal time – temperature relations (resistance) no

evidence has been obtained that the increased resistance lasts indefinitely. It therefore seems possible that acclimation may have little effect on the incipient lethal level, but this problem requires further investigation. For the purposes of this study, incipient lethal levels are considered to lie between 1° and −10° C. for *P. maniculatus* and between 1° and 10° C. for white mice, since these species could not be successfully acclimated to temperatures lower than this.

The time to death of an animal in the cold may be divided into two stages (*a*) the time during which normal metabolic rate and body temperature is maintained, and (*b*) the time during which body temperature and metabolism decline to lethal levels. An attempt has been made to distinguish between these two periods for *P. leucopus* (Fig. 2) using data obtained in a separate communication (13). In this species, metabolic rate was measured at low temperatures until it declined to levels at which the mice became torpid (body temperature 13°-18° C.). From data obtained on mice at a series of low temperatures, the periods during which normal metabolism was maintained (maintenance time) and that during which it declined (decline period) were determined. These decline periods have been subtracted from resistance time at each exposure temperature to give the broken lines shown in Fig. 2. Since the decline period changed relatively little with severity of test conditions (13), it composed a much larger fraction of the resistance time in short duration than in long duration tests. Subtraction of this period therefore results in a more uniform response of the mice to decrease in temperature. This derivation suggests that the log maintenance time varies directly with the exposure temperature and that the curvilinearity of the resistance curves is associated with the body cooling stage of the mice. The progressive increase in individual variability with increase in exposure temperature in *P. leucopus* (Fig. 1) also appears to be associated with the nonuniform increase in resistance time for the same increase in temperature.

Thermal acclimation is a factor of major importance in the stabilization of response to low environmental temperature. The only factor rigidly controlled in these experiments was temperature; yet good agreement in duplicate lots of deer mice was obtained in spite of variations of season, size, age, domestication, and to a certain degree, diet. Variations between groups of rats acclimated to the same temperatures were encountered, but even greater differences in previous history were present.

Although it is generally recognized that large animals are more resistant to cold, there appear to be only isolated observations concerning the effect of body size on resistance within the same species. Sellers *et al.* (21) noted that among animals not accustomed to cold, heavier rats appeared to live longer than lighter ones. Sealander (18) reported a similar trend for deer mice at low temperatures, but Horvath (16) indicated that heavier body weight was not always associated with increased survival of various species at low temperatures. No correlation between weight and time to death has been noted among rats and white mice studied by the writer, but, in deer mice, a small positive correlation was found.

Small but significant differences were observed in resistance of male and female white mice and deer mice. In deer mice (18) and rats (7, 19) on the other hand, sex is reported to have no effect on response to low temperatures.

Specific differences in cold resistance, at least among deer mice and white mice, appear to reflect differences in physiology, rather than differences in body size. Rats, which were over 10 times as large, were more resistant than mice, but the increased resistance was not proportional to the increased weight. The comprehensive study of Sealander (18) on acclimation in deer mice unfortunately cannot be compared with these results except at the lowest temperatures, because Sealander's mice were without food during exposure. At very low temperatures the mice die so quickly that they are unable to deplete available food stores in the gut, and hence they are not in a fasting state. Under these conditions death may be ascribed to temperature, but at higher temperatures the cause of death was at least partly due to starvation. The effect of acclimation on resistance time at very low temperatures appeared similar for deer mice studied by Sealander and by the writer, but these effects were small compared to those observed at higher temperatures when the animals had access to food and died after longer periods of time. The full benefits of acclimation apparently are not realized in animals deprived of food.

The finding of Adolph (1) that cold acclimation did not decrease the lethal body temperature of rats in a cold environment has been confirmed for white mice. In these animals there was no progressive decrease in lethal body temperature with decrease in acclimation temperature. However, deer mice and white mice acclimated to a high temperature (30° C.) died with a significantly higher average body temperature at death. It therefore appears that thermal history is able to influence the heat debt that can be withstood, but this influence may apply only for higher acclimation temperatures.

The physiological and morphological factors responsible for changes in cold resistance during acclimation are generally considered to involve alterations in metabolic rate and body insulation. Metabolic aspects of acclimation in deer mice are being reported subsequently (13). Insulative changes have received much less attention, but they have been the subject of several reports (15, 17, 18, 23, 24) indicating that temperature and season may change the thickness and density of the fur. However, there have been no measurements of pelage heat conductivity on animals acclimated to different temperatures.

In *P. maniculatus* no substantial differences in pelage insulation were found in warm and cold acclimated mice although the two groups differed greatly in cold resistance. These findings confirm observations reported elsewhere (13, 20) that increased cold resistance results largely from enhanced ability to maintain high heat production rather than by reduction of heat loss. Since the efficiency of insulation is low on objects of small diameter (5), not much protection is to be expected through increased thickness and density of fur in mice. It may be, however, that significant variation in insulation occurs with seasonal changes in nature. Further study is being made of this problem.

Acknowledgments

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ENERGY METABOLISM OF THE WHITE-FOOTED MOUSE, *PEROMYSCUS LEUCOPUS NOVEBORACENSIS*, AFTER ACCLIMATION AT VARIOUS ENVIRONMENTAL TEMPERATURES[1]

By J. Sanford Hart

Abstract

Acclimation of white-footed mice to successively lower temperatures (30° C., 20° C., 10° C.) had no effect on average initial oxygen consumption levels during exposure to environmental temperatures from 30° C. to − 26° C., but prolonged the time that the initial levels could be maintained. When the initial oxygen consumption could no longer to maintained, there was a gradual decline to the torpidity level. The duration of this metabolism decline period increased only slightly with increase in total duration of the test. The effect of acclimation on ability to maintain metabolism appears sufficient to account for its influence on resistance to low temperatures in this species.

Introduction

The resistance of rats (24) and mice (21) to low temperatures may be increased by previous exposure of the animals to cold. A detailed description of the effect of acclimation on cold resistance has been given above for several species (11), but the underlying physiological changes have not been investigated.

Two major factors may operate to change resistance to low temperatures. These may be broadly classed as insulative changes in which pelage insulation, surface–volume ratios, and circulation of blood may be altered, and metabolic changes, in which the capacity of the animal to produce heat may be altered. A considerable amount of evidence indicates that the metabolic rate at thermal neutrality may be changed following prolonged exposure of small animals to high or to low temperatures (1-8, 13, 14, 15, 20, 22), but few studies have dealt with the metabolic rate of nonfasted animals at temperatures in the lethal range. Sellers *et al.* (23) have shown that cold acclimation in rats is associated with an ability to maintain an increased production of heat rather than with a decrease in heat loss. The present study of *Peromyscus leucopus* is concerned with the extent to which changes in metabolic rate during acclimation may account for the observed changes in cold resistance.

Methods

White-footed mice, *Peromyscus leucopus noveboracensis* (Fischer), used in these tests were obtained from Tumblebrook Farm, Brant Lake, N.Y. Groups of mice, 6-8 weeks old, were placed in constant temperature rooms on June 7 at 10°, 20°, and 30° C. (± 1° C.). Food, light, and cage conditions during this period have been previously described (11).

On Aug. 8, three females and three males were withdrawn at random from each room for determination of oxygen consumption. The measurements

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were made over an eight week period, and the duration of each test ranged from 50 to 400 min. Except for certain runs at the end of this series the same six mice from each room were used in all tests. When not under test the mice were kept at the temperatures to which they had been acclimated.

Oxygen consumption was determined with a closed circuit system, with a liquid bath for temperature control and with mice placed singly in brass metabolism chambers as previously described (10). Before the mice were placed in the chambers, the system with chambers was precooled to the temperature of the liquid bath. This precaution permitted volume equilibrium in the system to be established rapidly (20-30 min.) after introducing the mouse, but initial readings were commenced immediately.

Temperatures were recorded from the chamber air and wall as previously described (10), and the average of these was taken as the environmental temperature. Average environmental temperatures and order of testing were 10.0, 30.0, 19.5, 0.0, − 11.2, − 15.5, − 21.0, and − 26.0° C. (± 2.0° C.).

At subfreezing temperatures some of the mice were unable to maintain their initial metabolism and determinations were made during the decline period. The metabolic rate of some mice started to decline almost immediately after they were placed in the chambers. Under these conditions initial readings were subject to considerable error because volume equilibrium in the system had not been established. The determinations were nevertheless of value in showing the time course of falling oxygen consumption. The mice were removed from the chambers in a torpid state, when their oxygen consumption had fallen to about 100 ml. per hr.

Body temperatures in torpid mice after removal from the chambers were recorded by thermocouples inserted to a depth of 2 cm. in the colon.

Results

Acclimation to 10°, 20°, and 30° C. did not modify average initial oxygen consumption at temperatures from 30° C. to − 26° C. (Fig. 1). The total range of variation, indicated by vertical bars, averaged 17.7% from the mean.

The body weights throughout the tests (Table I) averaged 21.4 gm. for the 10° C. mice, 25.4 for those at 20° C., and 26.2 gm. for the 30° C. mice. When the oxygen consumption of the mice was averaged by weight groups and the relative effect of increasing weight determined (Table II), it was found that an increase of 5 gm. raised the oxygen consumption average by about 3%. This effect is not large enough to influence significantly the results of Fig. 1.

The effect of previous acclimation on the maintenance of metabolic rate at − 11 and − 21° C. is illustrated in Fig. 2. Results at other temperatures were similar. At − 21° C. the initial oxygen consumption of mice acclimated to different temperatures varied between 300 and 400 ml. per hr., and at − 11° C. between 200 and 300 ml. per hr. Acclimation to lower temperatures was associated with extension of the time that the metabolic rate was maintained (compare 20° and 30° C. mice at − 21° C.) or with no decline at all

(10° C. mice, 20° C. mice at − 11° C.). Once metabolism began to decrease it usually fell gradually to approximately 100 ml. per hr. (metabolism decline period) when the mice were removed.

Body temperatures of torpid mice removed from the chambers ranged between 13° and 18° C. Observation of the temperatures, metabolic rates, and appearance of the mice at removal, indicated that 5 to 15 min. further exposure would have been lethal. Torpidity was not observed in any mice at temperatures above − 11° C.

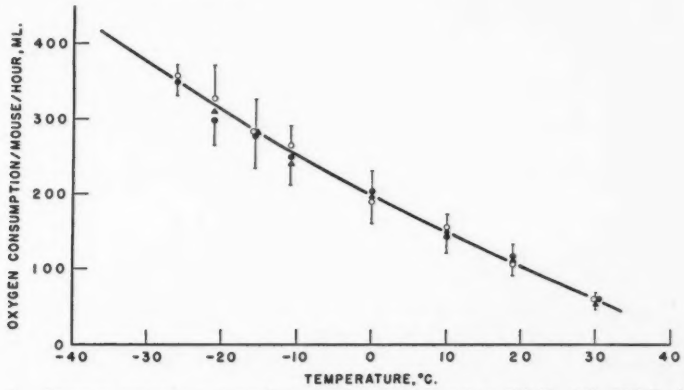

FIG. 1. Average oxygen consumption of mice acclimated to 10° C. (●), 20° C. (○), and 30° C. (▲) at different environmental temperatures. Vertical bars show range of variation.

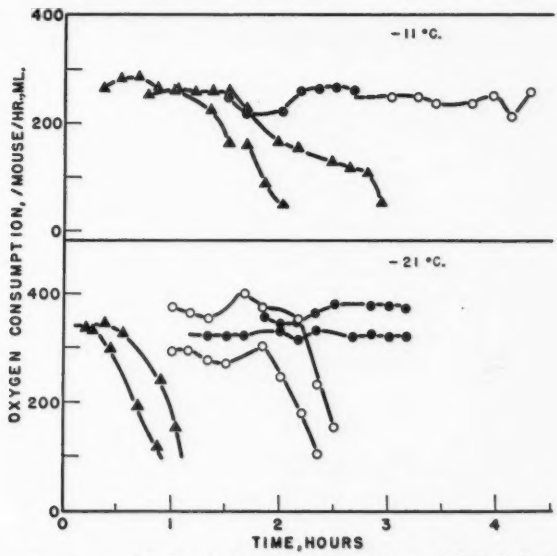

FIG. 2. Oxygen consumption in single mice acclimated to 10° C. (●), 20° C. (○), and 30° C. (▲) in relation to duration of exposure to − 11° C. and − 21° C.

TABLE I

AVERAGE BODY WEIGHTS OF MICE USED IN TESTS

Test temperature	Body weight, gm.		
	10° C.	20° C.	30° C.
30.0	21.0	25.6	25.6
19.5	22.	25.2	27.0
10.0	21.6	25.9	26.7
0.0	21.4	25.2	25.3
− 11.2	21.4	26.1	25.8
− 15.5	—	—	26.3
− 21.0	21.5	24.9	26.5
− 26.0	20.8	24.6	—
Average	21.4	25.4	26.2

TABLE II

EFFECT OF INCREASED BODY WEIGHT OF MICE ON AVERAGE OXYGEN CONSUMPTION AT DIFFERENT TEMPERATURES

Test temperature	Group A, below 23.9 gm.		Group B, 24 to 28.9 gm.		Group C, over 28.9 gm.	
	Average B.W.*, gm.	Oxygen consumption, ml. per hr.	Average B.W., gm.	Increase in oxygen consumption over Group A, %	Average B.W., gm.	Increase in oxygen consumption over Group A, %
30.0	19.9 (8)	56	25.5 (6)	3.6	30.4 (4)	3.6
19.5	20.7 (7)	109	25.3 (7)	1.0	29.9 (4)	3.2
10.0	20.7 (7)	142	26.0 (7)	3.5	30.7 (4)	9.0
0.0	19.6 (7)	198	24.5 (5)	0.0	30.0 (4)	0.7
− 11.2	20.8 (8)	245	24.9 (5)	3.1	30.6 (3)	5.5
− 15.5	20.0 (7)	274	24.5 (6)	4.7	30.0 (3)	3.6
− 21.0	20.9 (5)	296	25.6 (5)	7.4	31.3 (2)	19.0
Average	20.4		25.2	3.3	30.4	5.4

* *Average body weight for the number of mice indicated in parentheses.*

The average duration of the metabolism decline period increased from about 35 to 85 min. as the total time of the test increased from about 35 to 400 min. (Fig. 3). The decline fraction therefore decreased with increasing total time to torpidity. Differences in the decline periods due to acclimation were not apparent in these data; at comparable times to torpidity, the decline periods were similar. Therefore, it appears that acclimation changed mainly the time that metabolism was maintained (maintenance time) before the onset of the decline period.

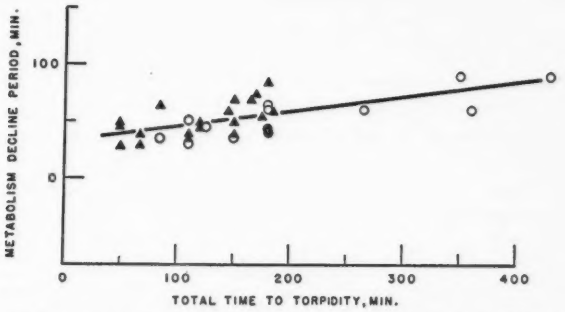

FIG. 3. Duration of metabolism decline period of mice acclimated to 10° C. (●), 20° C. (○), and 30° C. (▲) in relation to exposure time.

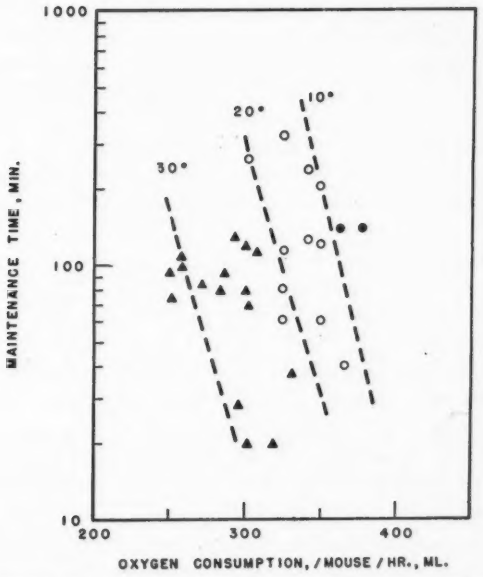

FIG. 4. The relation between maintenance time and oxygen consumption of single mice acclimated to 10° C. (●), 20° C. (○), and 30° C. (▲). Curves represent predicted relationship derived from other data. See text.

In Fig. 4 is shown the correlation between oxygen consumption and maintenance time for mice acclimated to different temperatures. Test temperatures ranged between − 11° and − 26° C. Results (broken lines) predicted from previous lethal temperature determinations (11) are compared with those for single mice in these tests. Predicted maintenance time was obtained by subtracting metabolism decline period (Fig. 3) from the resistance time (11, Fig. 3) at each temperature, and the corresponding metabolic rate at that temperature was obtained from Fig. 1. At each acclimation temperature, the predicted maintenance time decreased with increasing oxygen consumption. In spite of considerable variation it may be noted that the 20° C. acclimated mice were able to maintain higher metabolic rates (at a lower temperature) than the 30° C. mice for the same time. The 10° C. mice were able to maintain high metabolic rates for still longer periods and a decline was observed for only two animals, at the lowest temperature used (− 26° C.). Although observed rates averaged higher than predicted, the observed difference in rate between 20° and 30° C. mice was similar to the calculated difference (50-60 ml. per hr.) for the same maintenance time.

Discussion

Metabolic studies on acclimation of small birds and mammals have produced conflicting results. At temperatures below thermal neutrality, results on rats (3), hibernating animals during summer (5, 14), various birds (4), and rabbits (7) have shown that a decrease in acclimation temperature results in progressively higher heat production at the same exposure temperature. On the other hand differences in thermal history did not affect oxygen consumption of rats (1, 23) and hamsters (2) at the same low temperatures, nor did it affect the oxygen consumption of white-footed mice at any temperature. Prior fasting of the animals in certain of these studies (3, 4, 5, 7, 14), and not in others (1, 23), may well be the cause of these discrepancies, since Swift (25) has shown that fasting may change the metabolic rate of rats at different temperatures.

The finding that differences in thermal history do not affect oxygen consumption at the same exposure temperature indicates that white-footed mice may acclimate to changes in temperature only by changing heat production. In agreement with the results cited above (1, 2, 3, 4, 5, 7, 9, 14, 23), mice do not appear to become acclimated to low temperatures by reducing temperature sensitivity (18, 19). In other words, there is no evidence of increase in overall body insulation with acclimation to decreased temperature comparable to that found in larger mammals (12, 16, 17). Failure to find a significant influence of acclimation on pelage density (21) or pelage insulation (11) of mice further supports the metabolic findings.

Even at temperatures in the lethal range, thermal history did not affect the initial rate of oxygen consumption, but it did change the time that high metabolic rates could be maintained. In confirmation of Sellers' interpretation (23) for rats, maintenance time appears to be the principal factor responsible

for increased resistance time in white-footed mice. Also, when mice become acclimated to lower temperatures they are able to maintain higher metabolic rates for a given period and hence are able to decrease their resistance temperature (11).

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CELLS OF THE THYMUS AND THEIR RELEASE OF CYTOPLASMIC PORTIONS *IN VITRO*

I. THE METHOD OF RELEASE OF DISCRETE CYTOPLASMIC PORTIONS BY BASOPHILIC CELLS (CHANGED LYMPHOCYTES) FROM THE THYMUS *IN VITRO*[1]

By V. E. Engelbert[2]

Abstract

During culture experiments with thymic lobules of chick embryos and young rats, it could be observed directly that cytoplasmic fragments were released from lymphocytes that had changed in appearance. Any similarity to the cell appearance described below has been found only once in the literature, namely in an illustration by Watney from foetal calf thymus published in 1882; Watney however, neither labelled nor described the cells. The distribution of the cytoplasm in readiness for release could be observed when the cells came to lie against the side of the culture vessel. The long processes were then seen to be tubes formed by the cell membranes. A tube could be followed from its first appearance. At this stage it appeared empty but soon a discrete portion of cytoplasm would be seen emerging from the endoplasm and passing slowly into the tube, later followed by several others. The portions remained discrete and did not coalesce. The individual portions would eventually roll out into separate side-tubes. The end pieces twisted off and floated free when the tubes were supported only by the medium. The release of discrete cytoplasmic portions is not clasmatosis as that process is usually understood, nor a degeneration, nor yet a disintegration, but a function of living cells.

Introduction and Literature

The role and the potentialities of the lymphocyte which constitutes 20% or more of the white cells of the blood has not been settled yet, in spite of the attack from several different angles by many workers over a great number of years.

At this time, only one phase of lymphocyte behavior as reported in the literature will be dealt with; namely, the observance by many workers of pieces of cytoplasm or fragments of lymphocytes in sections of lymphatic tissue.

As early as 1912, Downey and Weidenreich (5) had shown that in sections of lymphatic tissue small pieces of cytoplasm were lying free near the lymphocytes. Weill (11) in 1913 showed, in sections of the thymus, cytoplasmic pieces lying near the "Thymusrindenzellen". The presence of lymphocytic fragments in cultured thymus was demonstrated by Popoff (7) and Tschassownikow (8), and Emmart (6) observed stages of disintegration of thymocytes. Fragments of lymphocytes in sections of the spleen are shown in Watzka's paper (10); Darcy (4) in a study of homografts did not observe the "pyronin cells" to "shed their cytoplasm", but many lymphocytes became pycnotic and sometimes fragmented during graft destruction.

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Contribution from the Department of Zoology, University of Toronto, Toronto, Ont. This research was supported by a grant-in-aid from the Banting Research Foundation.

[2] *Assistant Professor of Histology, Department of Zoology.*

Bergel (1, 2) found that lymphocytes from the thymus remained unchanged in cultures and finally degenerated. Bichel (3) claimed that cultured leukosis cells degenerated similarly.

Free pieces of cytoplasmic fragments have been observed by the author in many *in vitro* experiments involving splenic and thymic tissue of rats, mice, and chick embryos and of frog and fish spleen.

The results reported in this paper were obtained while the author was trying to observe the cells in the act of releasing cytoplasmic pieces. The cell behavior and appearance described below have, as far as the author knows, not been described before, and only two cells similar to the ones shown in this paper have been found in the literature, namely, in a section of calf thymus shown by Watney (9), who, however, neither labelled nor described these two cells. Watney made a careful study of the minute anatomy of the thymus using 10 mammalian species including the human, as well as four species of birds, three of reptiles, the frog, the axolot, the codfish, and the ray-fish. He shows in Fig. 49 (a camera lucida drawing of a section of thymus of a foetal calf) two cells that resemble closely two stages in the cell behavior reported in this paper, which the author has seen leading to the release of cytoplasm. Dr. Watney did not label nor describe those two cells, but drew them in such careful detail that we today, 70 years later, can interpret them on the basis of similar cells seen in the thymus of the rat and the chick.

Material and Methods

The present work was undertaken in order to study the so-called "shedding of cytoplasm" by lymphocytes. The thymus was chosen as donor tissue because of its easy accessibility, its size, and its relatively large population of lymphocytes.

The experiments carried out fall into two groups, the first consisting of 82 explants, 29 from chick embryos 13–14 days old and 53 from rats from 4 to 67 days old. Of the 82 explants, 46 were roller tube cultures, the rest hanging drop cultures, all in serum–saline media. The chicks were of healthy leghorn farm stock. The rats were all progenies of sib matings, the original stock of which (four litter mates, one male and three females) were bought from the Department of Biochemistry in the spring of 1951.

From this set of experiments sampling and subculturing was done at intervals during a culture period of less than a week. Cytoplasmic pieces could always be found as well as cytoplasmic granules, but one could not observe the cells that produced the cytoplasmic fragments and granules and it seemed imperative to know for certain what the cells looked like at the time that the pieces of cytoplasm were released.

A second set of experiments was therefore started with eventually 215 explants, 134 from chick embryos 13 to 20 days old and 81 from rats from 21 to 120 days of age. Lobules were carefully teased out from the lobes of thymi with the aid of a dissecting microscope. The lobules were cultured in serum–saline mixtures in a simple little shallow vessel. By using whole lobules the

tissue organization was maintained. The culture vessel consisted of a No. 0 cover slip placed on cover slip chips on an ordinary slide and sealed with paraffin. It was important to have this very shallow culture vessel, so that the cells would be confined to movements in lateral directions only and would have to stay within reach of focus of the high, dry objective.

The cultures were studied as soon as made and at intervals during the first 48 hr. Cultures were fixed every few hours up to 48 hr. and more. Methyl alcohol and iodine vapor were used as fixatives followed by staining with Giemsa alone or Giemsa plus pyronin. Hasting's blood stain was tried also. Fresh tissue was washed in glucosol, then teased in glucosol or Earl's saline so that a spread of cells was formed. The spread was then fixed and stained in the same way as the cultures. Imprints on dry slides of sectioned fresh thymi of chicks were also made and fixed immediately.

Release of Discrete Cytoplasmic Portions by Basophilic Cells

The different cells encountered in these experiments will not be discussed in this preliminary paper,—only the lymphocyte and its transitional first stages during which time the cytoplasm is released. These stages are met during the first 12 to 24 hr. of the culture period.

The transitional form or changed lymphocyte shows a less dense nucleus and a more basophilic cytoplasm than the original cell. The cytoplasm stains strongly with pyronin and one could call these cells "pyronin cells", as Darcy (4) does, without making further comparisons between the cells in these cultures and the cells of the homografts which Darcy studied.

The cells from the thymus of the chick embryos have been examined during a greater number of experiments than the rat thymus, but so far the observations from both these animals have corresponded well. The chick embryo provides such excellent material for thymus work that it is natural to work the problem through with it first.

The observations on *in vitro* preparations, both living and fixed, have corresponded well with immediately fixed fresh material.

In the chick embryo up to six thymic lobes can be found on either side of the neck, depending on the age of the chick. At 20 days the embryo presents several lobes that have undergone fatty involution.

From the work on the chick it seems that a cycle of activity (in relation to release of cytoplasm) can be observed since it is possible to find one lobule that is active right away, and one that becomes active in an hour or two, or still later. Such a cyclic pattern to this activity would be expected if the release of cytoplasm is a normal function of the vertebrate thymus, as these experiments seem to indicate. When the cells are in an active phase in the cultures one cannot find a field without them.

The changed lymphocyte soon shows a tubelike projection extending from the edge of the cell; this tube is actually formed by the cell membrane. At this time the basophilic cytoplasm shows a pattern indicating the discrete cytoplasmic portions that soon will be released. These portions enter the tube one at a time, slowly gliding out from the cell.

PLATE I

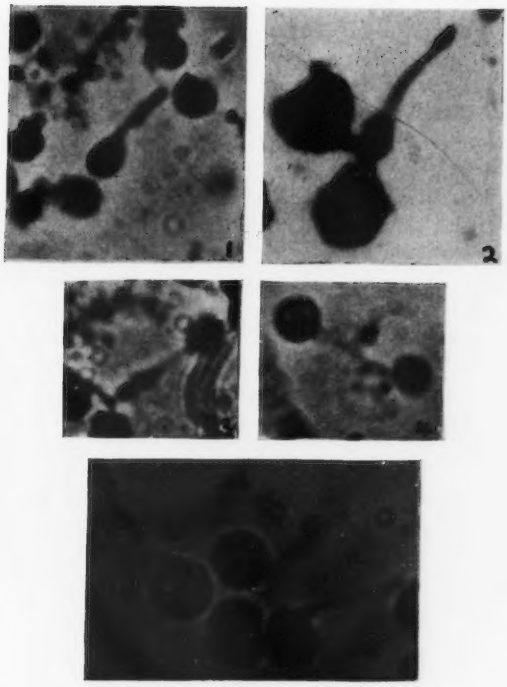


FIG. 1. Living cell in an eight-hour culture of fourth lobe of thymus of a 20-day chick embryo with one tubular extension containing one cytoplasmic portion.

FIG. 2. Fresh preparation of thymus from an 18-day chick embryo, made in glucosol and stained with Hastings blood stain.

FIG. 3. Living cell from same culture as Fig. 1, after eight and one-half hours culturing, showing four portions at end of tube seemingly coalescing.

FIG. 4. Living cell showing three cytoplasmic portions passing out into side branches of the main tube. Five-hour culture from third lobe of thymus from the same chick as Figs. 1 and 3.

FIG. 5. Living cells from the fourth lobe of thymus of a 15-day-old chick embryo cultured about 12 hr.

Photomicrographs. Leitz objective 45×, Leitz ocular 25× used for all the above except Fig. 2 where oil immersion objective (Leitz 2 mm. 90×) and Leitz 20× ocular was used.

The tubular structure of the projections can best be seen in cells that are flat against the glass of the culture chamber and one can then, as it were, observe the distribution in the tube of the individual cytoplasmic masses in "slow motion". If the tube lies free in the fluid medium it is in constant movement or the angle for observing it is vertical, or in the best cases, oblique. In the fluid it looks like a long thin thread with the cytoplasmic portions appearing as beadlike enlargements of equal size and evenly spaced (Fig. 9). It seems that the distances between the number two, three, and four portions are amazingly even and one wonders whether the "sliding out" of a new portion is due to more than chance or whether the interval between the release of two portions is a matter of chance. The interval between the first and second seems to be the longest. It has not been possible to photograph the threadlike tube in the fluid because of its movements, but an attempt to produce a cinematographic record will be made this winter.

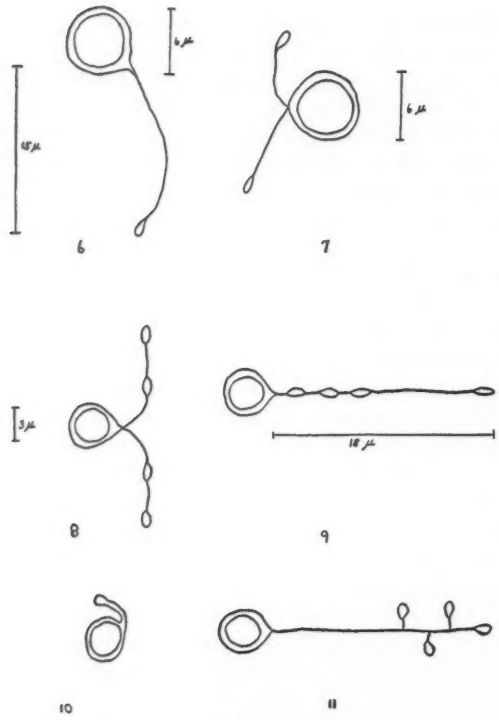
The first cytoplasmic portion to enter the tube glides eventually out to the end of the tube which now is about three cell diameters long (see Pl. I, (Figs. 1, 2, 5)). It is this stage that one of the two cells shown in Watney's Fig. 49 (9) represents. The other one that he drew is an earlier stage. When the first portion reaches the wall of the blind end of the tube, and if the tube lies free in the fluid medium, the now expanded end of the tube will start to whirl at a considerable speed. This whirling is no doubt due to surface tension forces in the shallow chamber, but it is so common and constant a feature in the "active" cultures, that these whirling pieces were named "spinners" in this laboratory. This term was useful while notes were being taken.

Four or more discrete portions of cytoplasm can soon be seen following the first one (Figs. 9, 11). Slowly, one at a time, they glide out into the tube, filling its diameter neatly. Sometimes it appears as if all the pieces eventually glide right to the end as if they were going to coalesce with the first one (Pl. I, Fig. 3), but after some minutes the individual pieces reappear as separate entities again, each passing out into individual small side-tubes (Fig. 11; Pl. I, Fig. 4).

Where the twirling end pieces lie free in the medium one can, the odd time, with careful watching see an end piece twist off and float away free, losing the fast twirling movement and just drifting or stopping against other cells or pieces.

Where the tubes lie against the glass surface, the tubes seem to break up eventually, thus releasing pieces.

A cell with a new tube can be seen in Pl. I, Fig. 2, which is a photomicrograph of a fresh noncultured preparation. Tubes can apparently come off from several places on the one cell. One cell was observed in a fixed preparation to have four evenly spaced processes as if they were in N.S., E.W. directions, all of the same length. A very common arrangement is a bifurcated tube where the two free ends are blind and rise from a single tube. The two ends will both twirl as in "callisthenic" exercise. Sometimes one tube will



FIGS. 6 AND 7. Outline drawings of cells from a 24-hr. culture of thymus from a 90-day-old rat.

FIGS. 8 – 11. Outline drawings of cells from a 12 hr. culture of the thymus from a 16-day-old chick embryo. FIG. 10. A later stage of the cell shown in Fig. 8 after one cytoplasmic portion has been slung off. FIG. 11. A later stage of the cell in Fig. 9.

Actual measurements made with Leitz ocular micrometer shown on figures.

twist off before the other one. Secondary portions can be seen on the two branch tubes both at the same distance from the cell (Fig. 8). The tubes are not pseudopods. The changing lymphocytes have normal pseudopods applied to the glass surface and often also characteristic processes with cytoplasmic end pieces sticking up in the fluid. This can be seen in cultures even when the cells are actively amoeboid.

The activity lasts several hours but if a culture is active in the early afternoon, it will usually show much debris next morning due to the free cytoplasmic portions. The culture medium seems to look like a "gruel". At this time a number of mononuclear cells have appeared and begun to phagocytose the free pieces which they eventually seem to clean up. These mononuclear cells soon dominated the culture.

Some cells seem to release most of their cytoplasm and these possibly die; others seem to release only part of their cytoplasm and then differentiate

further, possibly forming plasma cells. These cells, which also may come from other sources, will under certain conditions release their cytoplasm in large uneven portions that fragment further by repeating the process. It is possible however that the first release of the even-sized portions of cytoplasm is prevented under the culture conditions that make the plasma cells release their cytoplasm.

These problems, involving the plasma cells and other cell types, as well as the cytochemistry of the cells in the phases of releasing cytoplasm, will be dealt with in subsequent papers.

It would appear that these *in vitro* experiments, supported by cytological techniques, may throw light on the function of the vertebrate thymus as well as on the function and potentialities of the lymphocyte.

Acknowledgments

Some years ago Dr. Raymond C. Parker of the Connaught Medical Research Laboratories gave generously of his time and facilities to teach tissue culture technique to the author and has since given aid with advice and material whenever it was needed. The author wishes to express sincere gratitude for this fundamental help.

Dr. K. C. Fisher of this department made it possible to start the project of tissue culture by providing several of the large pieces of equipment as well as the necessary funds. For his interest and assistance it is a great pleasure to extend grateful acknowledgments.

To Miss Anne M. Cockeram, who has carried out the experiments and all the phases of technique with great care and a never failing interest, the author extends grateful appreciation of a most able co-worker.

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RATE OF GAIN AND LOSS OF COLD RESISTANCE IN MICE[1]

By J. Sanford Hart

Abstract

The resistance time of white mice acclimated to 10 and 30° C. was 1730 min. and 67 min. respectively when both groups were exposed to −17° C. After shifting the 30° C. mice to 10° C. and the 10° C. mice to 30° C., the resistance time of the original 30° C. mice at −17° C. became approximately the same in five weeks as that of the original 10° C. mice and vice versa. Change of resistance time was greatest in the first two weeks and it gradually declined thereafter. Rate of loss of resistance in the first week was greater than rate of gain in terms of resistance time at −17° C., but appeared slightly less than rate of gain in terms of resistance temperature. In white-footed mice, several short (two- to three-hour) exposures to cold increased cold resistance.

Introduction

It is generally recognized that resistance of animals to cold is gained or lost slowly and that gradual adjustment is one of the characteristic features of acclimation. However, there have been few attempts to measure the actual rate of change in physiological attributes of animals undergoing thermal acclimation. Gelineo has shown that the basal metabolic rate of rats may continue to change for periods ranging from 3 to 25 weeks following change of thermal environment (4, 5, 6, 7). However, Schwabe *et al.* (12) found that basal metabolism of rats underwent no further change after 15–30 days in the cold. During acclimation of rats to cold, thyroid activity (11), thyrotrophic activity of the pituitary (13), tissue ascorbic acid (3), peripheral blood circulation (2), and blood pressure (8) may continue to change for periods ranging up to four months.

Lethal temperature determinations on rabbits, rats, and mice (1, 9, 14) have shown that previous exposure to low temperature results in substantial increase in resistance to cold. In rats, development of this resistance was noticeably faster than its loss after removal from a cold environment (14). Further observations on gain and loss of resistance are presented for mice.

Methods

The environmental conditions during acclimation, details of testing, and methods of analyzing the results have been previously discussed (9). Adult white mice used in these tests were exposed for eight weeks to temperatures of 10° C. and 30° C. After this period, groups of six mice (three males and three females) from each temperature were exposed to −17° C. and the time to death of each mouse determined. On the same day, the remaining 10° C. acclimated mice were transferred to a 30° C. environment and the remaining 30° C. mice transferred to a 10° C. environment. After one week six mice from the warm (30° C.) room and six from the cold (10° C.) room were exposed

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to a temperature of −17° C. and the time to death determined. The same procedure was followed at weekly intervals for five weeks and the final test carried out on the seventh week. The change in resistance to cold was recorded as the change in mean logarithm of the time to death (resistance time) at −17° C. over the seven-week period (Fig. 1).

Additional data were obtained on two groups of six white-footed mice, *Peromyscus leucopus noveboracensis*, that had been held at 20° C. and 30° C. respectively for eight weeks and subjected to temperatures of −15, −10, +20, +30, −5, +10, and 0° C. in the stated order in running capacity tests (10) for two to three hours per day. Between exposures, the mice remained at their original acclimation temperatures. After completion of these exposures, the 20 and 30° C. mice were placed at −20° C. and −18° C. respectively, and the times to death determined.

Results and Discussion

The results of the experiment with white mice are shown in Fig. 1. The mice acclimated for eight weeks at 30° C. had an initial resistance time of 62 min. at −17° C., while the 10° C. mice had a resistance time of 1730 min. One week after the mice had been changed to their new environments, the

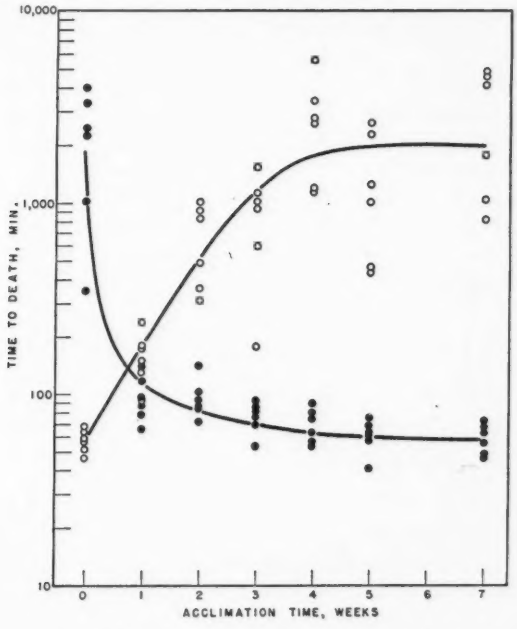

FIG. 1. Change in time to death of mice at −17° C. when acclimated at 30° C. (○) and placed at 10° C. for one to seven weeks, and when previously acclimated at 10° C. (●) and placed at 30° C. for one to seven weeks.

original 10° C. mice were less resistant than the original 30° C. mice and appeared to have lost most of their cold resistance. The gain in cold resistance of the original 30° C. mice after one week at 10° C. appeared to be distinctly less than the loss in resistance shown by original 10° C. mice. In the ensuing weeks there was a gradual loss of cold resistance by the original 10° C. mice and a gain by the 30° C. mice until, at the end of five weeks, the resistance time of the 10° C. mice was substantially the same as that of the original 30° C. mice and vice versa. These data indicate that the changes in cold resistance were completely reversible, and that rate of change of resistance is fastest in the initial stages of acclimation.

Additional results obtained with deer mice suggest that acquisition of cold resistance occurred when mice were exposed frequently to short periods of cold. In the group acclimated to 20° C. and killed at −20° C., it was anticipated from previous results (9) that the resistance time would be approximately 200 min., but four of the mice lived longer than 1500 min. In the group acclimated to 30° C. that were placed at −18° C., the anticipated resistance time was about 60 min. However, the times to death ranged from 55 to 160 min. (resistance time 100 min.). These findings are in keeping with

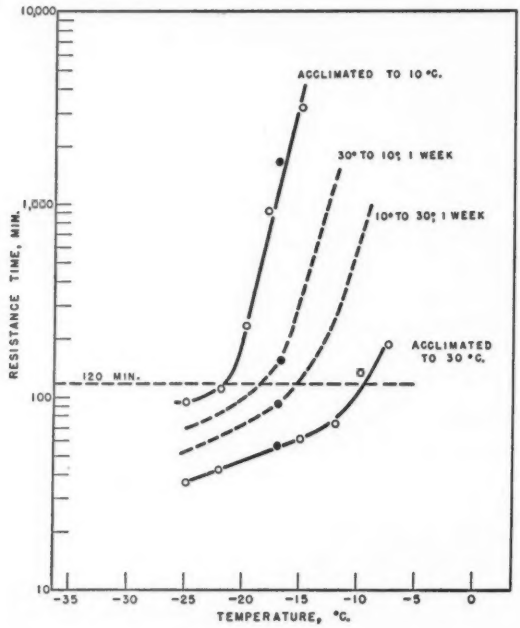

FIG. 2. Change of resistance times at different temperatures in mice acclimated at 30° C. and moved to 10° C. for one week and mice acclimated at 10° C. and moved to 30° C. for one week (●). Dotted curves hypothetical. Data for 10° C. mice and 30° C. mice (○) are previously reported (9).

observations on white mice that rate of change of cold resistance is rapid at first and then gradually declines as equilibrium with the environment is approached.

The interpretation of data on relative rates of gain and loss of resistance is complicated by the criterion used for measuring resistance. In terms of resistance time at −17° C. cold resistance appears to be lost faster than it is gained, but this observation might not be found in tests at a lower temperature or in terms of resistance temperature (temperature producing death in a given time (9)). This may be appreciated by examination of Fig. 2 in which resistance times of 10° and 30° C. acclimated white mice (9) at various temperatures (open circles) are shown, together with those for 10° and 30° C. mice at −17° C. before and after one week of changed environment (closed circles). Resistance time is not a linear function of temperature, and therefore the same change in temperature produces a much greater change in time along the "steep" than along the "flat" portion of the curve. In the tests at −17° C. it is therefore to be expected that loss of resistance in the first week will be faster than gain in the same period. On the other hand, if tests had been carried out at −25° C. little difference between gain and loss in the first week would have been expected.

The resistance temperature criterion appears to be of greater importance than resistance time from the biological standpoint. It is not possible to determine the change in resistance temperature within the first week of changed environment because tests were carried out only at −17° C. However, hypothetical curves (dotted) based on data for mice acclimated to 20° C. (9) have been drawn through the points representing resistance times of the interchanged mice at other temperatures. Using these curves and making comparisons at a fixed resistance time of 120 min. (horizontal broken line), the loss of resistance in the first week was about 7° C. (from −22° to approximately −15° C.) while the gain in the same time was about 9° C. (from −9° to approximately −18° C.). In contrast to resistance time, the decrease in resistance temperature in the first week appeared to be slightly less than the increase.

Comparison of rates of gain and loss of resistance from tests at a single temperature must therefore be treated with caution. For an adequate evaluation of these phenomena, it is necessary to determine resistance times at a series of temperatures after acclimation of animals for various periods of time.

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THE INFLUENCE OF THERMAL ACCLIMATION ON LIMITATION OF RUNNING ACTIVITY BY COLD IN DEER MICE[1]

By J. Sanford Hart

Abstract

Running capacity of mice acclimated to 10° C. was maximal from −5° to + 20° C. and was minimal at − 20° C. The capacity of mice acclimated to 20° and 30° C. was maximal from 0 to + 20° C. and + 10° to + 20° C. respectively. The lower limit for activity was reduced about 10° C. by a reduction of 20° C. in the acclimation temperature.

Introduction

Running activity of white mice is known to increase metabolic rate above the already enhanced level brought about by low temperatures alone (1, 2) and hence to impose a burden on the energy exchange in addition to that imposed by cold. If increased metabolic rate is a general consequence of increased activity in the cold, restriction of activity of small mammals at low temperatures (5, 8) will result in the conservation of heat.

Tests have been carried out to determine the influence of thermal history on running activity of deer mice at different temperatures, particularly its influence on limitation of activity by cold. These results have been correlated with changes in lethal temperatures produced by acclimation and interpreted in the light of previous metabolic findings (1, 2, 3).

Methods

Three groups of white-footed mice, *Peromyscus leucopus novaboracensis* (Fischer), were acclimated for 22 weeks to temperatures of 10°, 20°, and 30° C. under previously described conditions (4). Each group consisted of six adults, three males and three females. The mice were part of a series previously used (4) for lethal temperature tests and some had been used for metabolic rate determinations two weeks prior to the present study.

Maximum running speed (running capacity) was determined for each animal at temperatures from +30° C. to −20° C. in random order. Before each running test, the animal was left at the test temperature for two to three hours (averge two and one-half hours). All 18 mice were tested at a given temperature at approximately the same time. At all other times the animals were kept at acclimation temperature. The determinations were completed in 10 days.

Running tests were made in a revolving drum of 12 mesh wire cloth, 35.5 cm. i.d., with a baffle suspended from the shaft. Running capacity was determined by gradually increasing the speed of the drum until the mouse could not maintain its normal climbing position. The baffle stimulated the

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animal to maximum effort, but when the apparent limit to its capacity was reached, he was able to "ride" the drum, causing the baffle to rotate. The maximum speed attained without "riding" was taken as the running capacity. The determination was made relatively quickly, each mouse running only three to five minutes.

Results

The running capacities at different temperatures are shown in Fig. 1. Considering first the over-all effects of temperature on running, it may be seen that the highest speeds, averaging about 72 and sometimes exceeding 100 cm. per sec. (2.2 m.p.h.), were found between 0° C. and 20° C. At temperatures above 20° C. and below 0° to 10° C., the running capacities began to decline. Depending on the acclimation temperature, running capacities became minimal at temperatures between − 10° and − 20° C. At these temperatures some mice would not move in the drum, and the average was only 10 cm. per sec. (including all mice), or about one-seventh of the average maximal speeds.

The principal effect of cold acclimation was to increase running capacity at lower temperatures. Mice acclimated to 30°, 20°, and 10° C. showed an appreciable decline in running capacity at temperatures of 0°, − 5°, and − 10° C. respectively, and capacities were severally limited at − 10°, − 15°, and − 20° C. respectively. The limiting temperatures for running, obtained

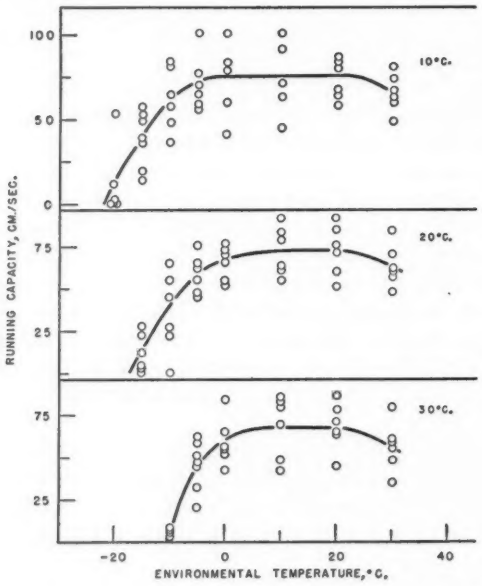

FIG. 1. Running capacity in relation to environmental temperature in mice acclimated to 10°, 20°, and 30° C.

by extrapolation to zero speed (Fig. 1), were approximately − 12° C. for 30° C. mice and − 22° C. for 10° C. mice; a difference of 10° C. The 30° C. acclimated mice were slightly inferior in running capacity to the 20° and 10° C. mice in the temperature range in which capacities were maximal.

Discussion

The enhancement of ability to be active at low temperatures appears to be one way in which acclimation may be of direct benefit to mice under winter conditions when problems of food procurement and self-preservation may be critical (6, 7). These tests indicate that cold acclimation permits full activity down to about − 10° C. Winter trapping operations by the writer indicate that deer mice may be active at even lower temperatures.

It may be asked whether limitation of running activity by cold is associated with impending lethal effects of these temperatures. The severe limitation of running in the 30° C. acclimated mice by a two to three hour exposure to − 10° C. is not surprising when it is recalled (4) that their resistance time is about four hours at this temperature. On the other hand similar limitation of running in the 10° C. acclimated mice occurred at a temperature of − 20° C., while the resistance time at this temperature was approximately 70 hr. In other words, temperatures that severely limit activity will eventually cause death. However, since limitation of activity by cold begins at temperatures 10° to 15° C. higher than those which restrict running to a minimum (Fig. 1), it is apparent that limitation begins at higher temperatures than those at which lethal effects were observed.

The limitation of activity by cold and the modifying influence of acclimation appears to be correlated with the ability of the mice to produce heat. It is known from results with white mice that activity increases heat loss and metabolic rate at cold temperature (1, 2) and it is probable than this applies also for white-footed mice. If, therefore, the animal is unable to produce sufficient heat to replace that which is lost during activity, body temperature might be maintained during abstention from activity. The decline of running speed to zero at low temperatures suggests that heat production during minimal activity is close to maximum. Since cold acclimation enabled the mice to produce more heat for a given period of time (3), it is to be expected that the thermal limits for running would be extended to a lower temperature.

The temperatures necessary for limitation of running activity may be regarded as a useful index of degree of acclimation in mice and possibly other small animals, but it is not known to what extent this may apply to larger mammals. Information on this subject is also limited by lack of data on body temperatures and metabolic rate of mice at limiting temperatures. Further studies correlating acclimation temperature and running capacity should also eliminate or correct for errors imposed by gradual acclimation to cold during the course of the tests.

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SURVIVAL OF *MACROCENTRUS ANCYLIVORUS* ROH., A PARASITE OF THE ORIENTAL FRUIT MOTH, ON DIFFERENT CONCENTRATIONS OF VARIOUS SUGAR SOLUTIONS[1]

By D. P. Pielou[2] and R. F. Glasser[3]

Abstract

Newly emerged adults of the hymenopterous parasite *Macrocentrus ancylivorus* Roh. were fed for their entire adult lifetime on a diet consisting solely of a solution, of one sugar only, of specified concentration. Sexes were kept distinct. Temperature was constant at 26.5° C. throughout the experiments. Dextrose, levulose, galactose, maltose, sucrose, and lactose were tested at seven concentrations ranging from 0.1% to 40%. The mean length of life on lactose at any concentration was only slightly longer than on water. Survival on galactose was moderate. Survival was satisfactory on all the remaining four sugars. The mean length of life on these sugars increased rapidly as concentration was increased and was optimal at 5%. Above this concentration survival declined, especially at high concentrations of 20% and 40%; however, at the latter concentrations survival was greater than at concentrations of 2.5%. Survival on a 10% honey solution was not as great as on solutions of the four sugars at the same concentration. The mean length of life of males generally was slightly less than that of females.

Introduction

The reasons for the work described in this paper are threefold. First, in extensive experiments on the selection for resistance to DDT in *M. ancylivorus* (Pielou and Glasser (6)), 10% sucrose was used as a survival diet in tests. It has been suggested that the mean life recorded would probably be greatly increased if honey were used instead; and this would allow more delicate estimation of selection effects because control mortality would be reduced. Secondly, it was desired to determine what sugar solutions promoted survival most effectively, with the view to the possible use of these adjuncts to naturally occurring foods of *M. ancylivorus*, if and when the special stocks of this parasite of the oriental fruit moth were released on a large scale in peach orchards. Thirdly, the information was required as a basis for behavior studies of the gustatory responses of the parasite to sugars and sweet substances.

Methods

Adults of *Macrocentrus ancylivorus* were reared on larvae of the potato tuber moth, which served as hosts, by the method of Finney, Flanders, and Smith (2). Those that had emerged on the day on which the experiment was begun were separated according to sex and placed in half-pint (234 ml.) bottles at a density of 50 per bottle. The insects were manipulated while they were stupified with carbon dioxide gas. The bottles were closed with standard milk bottle caps of waxed card. The nutrient solutions were supplied as follows. Each

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cap was perforated with a hole 3/8 in. in diameter and into this was squeezed a piece of dental cotton roll half an inch in diameter and two inches long with the bulk of the roll projecting into the bottle. Previously this dental cotton roll had been soaked in the solution under test; suspended from the cap, it provided the only source of water and food for the insects. The rolls and caps were replaced daily. A white filter paper 6 cm. in diameter was placed at the bottom of each bottle; the dead insects, falling on to this, could be seen easily and were counted daily until the population in each bottle was extinct. In each test 500 insects of one sex, or 10 bottles, were used. Tests on each sex were recorded separately. Six sugars at seven concentrations were used. The sugars were the hexoses: *d*(+) dextrose, *d*(−) levulose, and *d*(+) galactose; and the disaccharides: *d*(+) sucrose, *d*(+) maltose, and *d*(+) lactose. Concentrations used were spaced logarithmically at 0.1%, 1.25%, 2.5%, 5.0%, 10%, 20%, and 40%. These are expressed on the basis of grams of sugar per 100 ml. of water. Expressing concentration in percentages is more convenient than expressing it in terms of molar strength (3) because both monosaccharides and disaccharides were used. A 0.1 molar solution of maltose, for instance, has as much nutritive material as 0.2 molar solution of dextrose. All solutions were made in distilled water. Control data were obtained with a solution of honey adjusted to a 10% concentration of total sugar. All work was carried out at a temperature of 26.5° C. In all the definitive experiments about 44,000 insects were used.

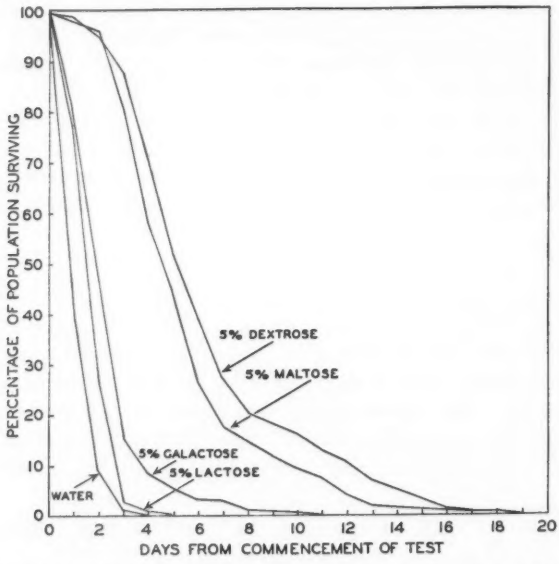

FIG. 1. Population decline of adult females of *Macrocentrus ancylivorus* fed solely on 5% solutions of the sugars indicated. The curves for levulose and sucrose are not shown but lie between those for maltose and dextrose. Each curve is drawn from data on 500 insects.

Results and Discussion

An example of the decline of populations is shown in Fig. 1, in which survival of females on 5% solutions is compared with that on water. The curves for sucrose and levulose come between those for maltose and dextrose. The results for the whole series of experiments, however, would show a confusing and large number of graph lines and are better summarized in a table (Table I) showing the *mean life* of females, in days, for the six sugars and seven concentrations. On water alone the mean life in days was 1.02; on 10% honey solution, 3.86. The figures for males are essentially similar but survival figures were a little lower, e.g., on 5% dextrose survival was 5.41 days compared with 6.23 for females.

Table I shows that lactose has practically no nutritive value; survival is but little better than on water alone. Hasset (3) working on *Drosophila melanogaster* Meig. found that some concentrations of lactose solution gave lower survival than water alone. For the honeybee adult (5) lactose has no nutritive value and in the larva (1) survival is very slightly better than on water alone as in *M. ancylivorus*. Galactose allows a moderate survival of *M. ancylivorus* at higher concentrations. It is interesting to note that in the honeybee adult (5) galactose allows no greater survival than pure water but that in the larva (1) survival is moderate as in *M. ancylivorus*. *Drosophila melanogaster* adults (3) also show such limited survival. The response of *M. ancylivorus* adults to lactose and galactose is therefore very close to that of the honeybee larva, is close to that of *Drosophila* adults, and diverges from that of honeybee adults as regards the latter sugar. On the remaining four sugars, dextrose, levulose, sucrose, and maltose, survival is almost equally great. The optimal concentration is 5%. Below this concentration survival falls off rapidly; above, it falls off slowly but distinctly. Although Hasset (3)

TABLE I

MEAN LENGTH OF LIFE IN DAYS OF ADULT FEMALES OF *Macrocentrus ancylivorus* ROH. FED ON SOLUTIONS OF DIFFERENT SUGARS OF VARIOUS CONCENTRATIONS

Concentration / Sugar	0.1%	1.25%	2.5%	5.0%	10.0%	20.0%	40.0%
Dextrose	1.56	2.25	3.27	6.23	5.04	3.96	4.05
Levulose	1.58	2.40	2.66	5.49	4.59	3.76	4.63
Galactose	1.49	1.71	1.17	2.06	3.75	3.47	3.38
Sucrose	1.52	2.71	3.63	6.09	4.57	3.93	3.97
Maltose	1.38	1.74	2.47	5.09	4.13	3.91	3.48
Lactose	1.60	1.56	1.58	1.58	1.47	0.93	1.22

does not draw attention to the fact that the same relation is apparent, his tabulated data indicate that for *D. melanogaster*, at least with sucrose solutions, mean life is greatest with $\frac{1}{10}$ *M.* solutions (3.42%) and less, both above and below this concentration. These facts stress the great importance of standardizing and maintaining concentration of food solutions in quantitative work on survival.

The results show that there is no great virtue in honey as far as longevity is concerned; in fact, the mean life on 10% sucrose is 4.57 days compared with 3.86 days on 10% honey. For large scale applied work with very large numbers of insects, 5% sucrose (cane sugar) solution seems to be the most economical. However, it does not follow that in nature *M. ancylivorus* would take this fluid in preference to others that might be less effective. In our experiments the insects had no choice but to feed on what they were offered. Preliminary evidence obtained in other studies by one of us (D.P.P.) shows that *M. ancylivorus* will take honey in preference to sucrose although survival is greater on the latter. Also, instances are known (4) where insects have a lower threshold of taste response, and therefore presumably greater preference, at this concentration, for a non-nutritious sugar than for one with high nutritional value.

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SOMATIC CHROMOSOMES OF HIGHER DIPTERA

I. DIFFERENTIATION OF TACHINID PARASITES[1]

By J. W. Boyes[2] and A. Wilkes[3]

Abstract

A method of analysis of somatic chromosome complements in cells of the brains of larvae and pupae is presented. Tachinid species have regularly 12 chromosomes in their somatic complements. These consist of five metacentric pairs and a pair of sex chromosomes which are acrocentric in most species. Careful measurement, on drawings, of each arm of each chromosome provides a basis for calculating the percentage that each pair constitutes of the total complement length and for determining the ratio of the long to the short arm for each pair. Results of analysis and other distinguishing features of the chromosome complements are presented for *Aplomya caesar*, *A. mitis*, *Ceracia dentata*, *Ceromasia auricaudata*, *Drino bohemica*, *Eumea westermanni*, *Lydella grisescens*, *Madremyia saundersii*, *Mericia ampelus*, *Nemorilla pyste*, *Neophorocera hamata*, *Omotoma fumiferanae*, *Phryxe pecosensis*, *Spathimeigenia* sp., *Winthemia datanae*, and *W. occidentis*. Most of these species can be distinguished by differences in the morphology of their chromosomes.

Introduction

At the Belleville laboratory many species of parasitic flies are imported from different parts of the world for use in the suppression of destructive insect outbreaks. Collections of imported parasitized hosts are reared under quarantine conditions, the parasites are identified, and those considered of economic value as biotic agents are either propagated in the laboratory or released directly in the field. The effectiveness of the parasites in suppressing increases in the numbers of the pests is later assessed by determining the frequencies of each parasite species in a unit population of the host, samples of the host population being usually taken during larval or pupal development. Accordingly, it is necessary to identify the parasites in their immature form or rear them to the adult stage for identification. The latter method does not provide a reliable means of determining the level of parasitism, however, since mortalities of the hosts and parasites during rearing in the laboratory are not only high but must also differ considerably from what they would be in the field. Although some progress has been made in identifying the parasites on the basis of morphological characters of the immature stages, this is not always reliable and hence the usefulness of differences in chromosome complements in identifying species was investigated.

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Adults of introduced parasites are sometimes very difficult to identify as indeed are endemic species that closely resemble the exotic forms. A more reliable means of distinguishing them is therefore of utmost importance if an appraisal is to be attempted of the control value of the imported species. Use of chromosome complements may provide certain means of identification and thus be of appreciable practical value.

Studies of chromosome complements may also be of value in other fields. The systematics of the more highly specialized Diptera are both complex and very difficult to use. A generally acceptable system of classification based on adult morphological characters has not as yet been advanced, and although the broad lines of evolutionary sequence of forms have been indicated, details are obscure. The results to be reported here and in papers in preparation are of value in these broader fields of biology and the fundamental problems in taxonomy.

TABLE I

TACHINID PARASITES STUDIED, THEIR HOST SPECIES, AND SOURCES OF PARASITE BREEDING STOCK

Parasite	Host
Aplomya caesar (Ald.)	*Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) and *Pyrausta nubilalis* (Hbn.) (Lepidoptera: Pyralididae)
Aplomya mitis (Mg.)*	*Cacoecia rosana* L.* (Lepidoptera: Tortricidae)
Ceracia dentata (Coq.)	*Melanoplus* sp. (Orthoptera: Locustidae)
Ceromasia auricaudata Tns.	*C. fumiferana*
Drino bohemica Mesn.	*Diprion hercyniae* (Htg.) (Hymenoptera: Tenthredinidae)
Eumea westermanni (Zett.)*	*C. rosana**
Lydella grisescens R.D.	*P. nubilalis*
Madremyia saundersii (Will.)	*C. fumiferana*
Mericia ampelus (Wlk.)	*Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae)
Nemorilla pyste (Wlk.)	*C. fumiferana*
Neophorocera hamata (A. and W.)	*Neodiprion lecontei* (Fitch) (Hymenoptera: Tenthredinidae)
Omotoma fumiferanae (Tot.)	*C. fumiferana*
Phryxe pecosensis (Tns.)	*C. fumiferana*
Spathimeigenia sp.	*N. lecontei*
Winthemia datanae Tns.	*Pseudaletia unipunctata* (Haw.) (Lepidoptera: Noctuidae)
Winthemia occidentis Rnh.	*Lambdina somniaria* (Hulst) (Lepidoptera: Geometridae)

* *Breeding stock from Europe; all others from Canada.*

The parasite larvae and pupae used in the study were collected and/or reared from laboratory stocks at Belleville under the direction of Wilkes. Technical assistants from both McGill and Belleville made many of the microscopic preparations. Other preparations and all of the cytological analyses of the chromosomes were made by Boyes at McGill. Identification of the insect specimens was checked by officers of Systematic Entomology, Division of Entomology, Ottawa.

Though the studies have included many representative species of three families of Diptera, including muscids, sarcophagids, and tachinids, the present paper deals only with the work on the Tachinidae. The species studied, their host insects, and the original sources of breeding stock are given in Table I.

Literature Review

Considerable literature has now accumulated on the somatic cytology of higher Diptera. Papers that contain descriptions of the somatic chromosome complements of species of Anthomyiidae, Calliphoridae, Sarcophagidae, Tachinidae, and Muscidae, which are all families in the Calyptratae, are most pertinent to the present studies and to others to be reported later. Accordingly, although there is considerable information available on the somatic cytology of other families, this brief review is restricted to the families mentioned above and will serve for the present and later papers of this series.

As early as 1908 Stevens (10) had studied the chromosomes of three species of higher Diptera from the subfamily Muscinae. She clearly described and figured 12 chromosomes (five metacentric pairs, plus a large, heteromorphic sex pair) in the spermatogonial mitoses of *Musca domestica*. Also, six pairs of chromosomes were found in *Calliphora vomitoria*, including a distinctly heteromorphic pair of smaller size than in *Musca domestica*. Unfortunately, spermatogonial mitoses were not found in *Lucilia caesar*, but six pairs of chromosomes, including a very large "heterochromosome" bivalent, were figured in a spermatocyte. Her studies included one sarcophagid species, *Sarcophaga sarraciniae*. Here also, six pairs of somatic chromosomes (including a pair of "heterochromosomes") were found and figured. Examination of the figures presented leads to the conclusion that, aside from the heteromorphic pairs (and with the possible exception of another pair in *Lucilia caesar*), all the chromosomes of these four species are metacentric. It is noteworthy that the larger heterochromosome (presumably the *X*) of *Sarcophaga sarraciniae* is shown with a terminal kinetochore in one anaphase (Fig. 29 of Stevens' Plate II). Unfortunately, primary constrictions were only shown as slender regions in her drawings so that no accurate analyses of relative arm lengths can be made. She reported six bivalents in the "first spermatocytes" of *Phorbia brassica*, including an "unequal pair of heterochromosomes", though no somatic complements were figured for this species of the Anthomyiinae.

Metz (4) published a very comprehensive study of the somatic chromosomes of Diptera, which included about 80 species representing 35 genera and 15 families. He used oogonial and spermatogonial tissues. The species that he studied included:

Anthomyidae
- *Homalomya* spp.
- *Fucellia marina* Macq.
- *Ophyra leucostoma* Wied.

Muscidae
- *Calliphora viridescens* Desv.*
- *Calliphora erythrocephala* Meig.
- *Musca domestica* L.
- *Muscina stabulans* Fall.*
- *Phormia regina* Meig.
- *Lucilia sericata* Meig.*
- *Pseudopyrellia cornicina* Fabr.*

Sarcophagidae
- *Sarcophaga falculata* Pand.
- *Sarcophaga tuberosa serraceniae* Riley
- *Sarcophaga dalmatina* Schin.
- *Sarcophaga bullata* Park.
- *Ravinia communis* Park.*
- *Ravinia peniculata* Park.

(*Note:* According to the Unit of Systematic Entomology, Ottawa, *Fucellia marina* Macq. = *Fucellia maritima* (Hal.); *Sarcophaga tuberosa serraceniae* Riley = *Sarcophaga sarraceniae* Riley; *Ravinia communis* Park. = *Euravinia therminieri* (Desv.); *Ravinia peniculata* Park. = *Ravinia pusiola* (Wulp.).)

The diploid chromosome numbers were reported as 12 for each of these species, but those marked with an asterisk were not figured. With reference to the Muscidae he stated, "Likewise the other Muscidae studied (Muscina stabulans, Calliphora viridescens, Lucilia sericata, and Pseudopyrellia sp.) agree with those already mentioned." Presumably this agreement included number, but in one case (*Muscina stabulans*) we have found only 10 chromosomes and since Metz was primarily concerned with the pairing association one cannot be quite certain that he definitely meant agreement in number. Similarly, concerning the Sarcophagidae he stated, "Several species of Sarcophaga have been used in this study and have been found to agree so completely in respect to chromosome behavior that they will be treated as a whole." Here also there is some doubt regarding chromosome numbers. This doubt is removed in a later paper (5), in which he states "all my Sarcophaga material, including several identified species, shows essentially the same (normal) chromosome group throughout." The name of one species (*Sarcophaga tuberosa serraceniae*) suggests that it may be the same as *S. sarraciniae* studied by Stevens but their complements differ. The chromosome number of *Ravinia peniculata* is clearly 12 from the complements illustrated.

In the same paper Metz (4) stated that the chromosome group of *Homalomya* sp. "is practically indistinguishable from that of Calliphora". His figure of anaphase in a second spermatocyte shows five metacentric pairs and a large acrocentric pair. His other figures are not convincing, except for pairing, with respect to his claim that "The chromosome group and the pairing phenomena of Ophyra are practically the same as those of Homalomya and Fucellia." He showed six metacentric pairs in *Musca domestica* and five metacentric pairs plus a small pair of *X*-chromosomes in *Calliphora erythrocephala*. He found that the chromosomes of *Phormia regina* "differed in no

essential respect from those in Calliphora and Musca" but here he probably referred to behavior only, since the *X*-chromosome in *Phormia regina* is distinctly smaller than in *Musca domestica* but larger than that in *Calliphora erythrocephala* in his drawings. The chromosomes of *Ravinia peniculata* were "essentially like those of Sarcophaga", in which five metacentric pairs and a small heteromorphic pair of sex chromosomes were figured. In his Fig. 95 of a somatic anaphase in *Sarcophaga* sp. the *X*-chromosomes were shown as acrocentric. Since kinetochores are not discernible in most of the somatic chromosomes in his illustrations, detailed analyses, as used in the present study, are not possible.

The somatic chromosome complement from an ovary of an unnamed species of *Sarcophaga* was figured by Metz (5) in 1922. The 12 chromosomes consisted of five metacentric pairs and a small heteromorphic pair. He showed prophase association of all four homologues in tetraploid somatic cells of the ovary. Frolowa (2) also resported on polyploid cells in Diptera and figured the somatic chromosomes of *Calliphora erythrocephala* Meig. and *Pegomyia geniculata* Bouché, both of which she listed as muscids. She noted prophase (and metaphase) associations of four and eight homologous chromosomes in tetraploid and octoploid somatic cells, respectively. Her figures of the chromosomes of *C. erythrocephala* confirmed previous observations as follows, "Wir sehen 5 Paare V-förmiger Autosomen und das 6. Paar kleiner stäbshenförmiger Geschlechtschromosomen, das y-Chromosom etwas kleiner als das x-Chromosom (Textabb. 10A)." Concerning the somatic complement of a female of *Pegomyia geniculata*, shown in her Fig. 12, she stated, "Das allgemeine Aussehen des Komplexes erinnert an den von *Calliphora erythrocephala*; wir sehen 5 Paare V-förmiger Chromosomen und 1 Paar kleiner Stäbschen, die wahrscheinlich Geschlectschromosomen sind." The kinetochores of the chromosomes of *P. geniculata* (which include three submetacentric pairs) are fairly clear for most chromosomes in Frolowa's Fig. 12*A*. Detailed analysis will be discussed in a later publication.

Studies of spermatogenesis by Keuneke (3) included some observations on the somatic chromosomes of the following species of higher Diptera listed as Muscidae: *Musca domestica* L., *Calliphora erythrocephala* Meigen, *Lucilia caesar* L., and *Sarcophaga carnaria* L. He found six pairs of chromosomes in *C. erythrocephala*, of which four pairs appear to us to be metacentric, one pair possibly acrocentric, and a small heteromorphic pair apparently acrocentric also. Similarly in *L. caesar* he found six pairs, including a small heteromorphic pair and five pairs that appear to be metacentric though he may have regarded his pair V as having terminal kinetochores. His observations on number and morphology of the chromosomes of *Musca domestica* seem to confirm those of Metz (4) but his *X*-chromosome is much smaller. Twelve paired chromosomes were also found in *S. carnaria*, including a heteromorphic pair in which the two members differ greatly in size. Details of morphology are not clear for the other pairs.

The somatic chromosome number of 12 for *Calliphora erythrocephala* was again confirmed by Naville (6). He stated, "Durant la division des cellules de revêtement de l'ovariole, on observe six pairs de chromosomes: cinq paires de grands éléments et un paire de courts bâtonnets chromatiques. Cette dernière paire constitue, comme l'a montré Keunecke, les hétérochromosomes X." His drawing shows five large metacentric pairs and a small heteromorphic pair, one member of which is about twice as long as the other. He has drawn the "Plaque équatoriale de cinèse goniale femelle de *Phormia terraenovae*" as Figs. 38 and 39. Twelve chromosomes can be counted with reasonable certainty, including two pairs that are metacentric, three pairs that are apparently submetacentric, and a small pair (presumably *X*-chromosomes) that appear to be metacentric. Although the kinetochores of the chromosomes in this species are not clearly indicated, apparently the longer arms approach twice the length of the shorter arms in three of the larger pairs.

Smith (8 and 9) has published photographs of the somatic chromosomes of two species of parasitic Diptera. In his 1943 paper there is an illustration of the somatic chromosome complement from the mesenteron of *Bessa selecta* (*?*). There are five metacentric to submetacentric pairs plus what is probably a pair of small *X*-chromosomes and a tiny dark fragment that might be an artifact. The later paper (9) included figures of the somatic chromosomes of *Neophorocera hamata* (*Phorocera hamata*), presumably from spermatogonial cells. Five pairs of metacentric to submetacentric chromosomes and a small heteromorphic pair of sex chromosomes were figured. This is the only reference to the chromosomes of any species of the Tachinidae found in the literature. The kinetochores are discernible in his photographs and approximate analyses of arm ratios and percentages of total complement length by methods outlined below have been attempted. These analyses will be referred to later.

The recent comprehensive study of spermatogenesis in *Musca domestica* by Perje (7) contained many valuable references to the earlier literature on the cytology of higher Diptera. Her findings confirmed the presence of 12 somatic chromosomes, including five metacentric pairs and a heteromorphic pair of sex chromosomes "consisting of a long metacentric *X* chromosome and a small rod- or comma-shaped *Y* chromosome".

Techniques

The present work has so far been limited to the somatic chromosomes of the "brain" ganglia, in most cases from third instar larvae. Salivary chromosomes were not suitable for study without extensive preliminary work: in many of these Diptera they have complicated connections and do not separate in squash preparations as they do in *Drosophila*. Plate I, Fig. 1, shows this situation in *Drino bohemica*. Fortunately the mitotic chromosomes of the brain are much larger than in the fruit flies and have proved to be satisfactory. Of course, a knowledge of the somatic chromosomes is essential before an interpretation of salivary chromosome arrangements can be attempted.

It is desirable to know which developmental stages have the most mitotic figures. Although we have still much to learn in this connection, present experience clearly indicates that larvae in the first half of the third stadium and those entering pupation are most satisfactory. During these periods the larger cells of the brain are more frequently found in mitosis and mitotic figures are generally more plentiful than at other stages. Usually the larvae were left in the host until they emerged of their own accord. Sometimes they were left until they showed reduced activity as they neared pupation or until a slight deepening of color indicated that pupation had started. After pupae have turned a dark reddish brown, few divisions are found and the chromosomes are usually so small that analysis is difficult or impossible. The stage selected for dissection is particularly important, however, since with rare specimens it is very disconcerting to find an otherwise satisfactory preparation in which mitoses are nearly absent and none can be studied. Satisfactory methods of artificially inducing mitosis would be useful.

Dissection and the Preparation of Microscope Slides

The larvae and young pupae were rinsed on a paper towel with a few drops of 0.74% solution of sodium chloride. This removed the slime and dirt that normally adhered to them. They were then placed in a fresh drop of saline on a glass slide. The head was pulled off, sometimes with the help of an incision several segments behind the mouth parts, though this is not essential. The brain is easy to locate and the only essential feature of the operation is speed. With practice this operation can be completed in less than one minute and the brain transferred directly to a drop of a modified Kahle solution for exactly one-half minute of prefixation before staining in iron acetocarmine. This brief prefixation seems to be sufficient to hasten the penetration of the stain. The formula for the modified Kahle solution is as follows:

Ethyl alcohol (95%)	—	15 ml.
Formaldehyde	—	6 ml.
Glacial acetic acid	—	1 ml.

Another modification used consisted of:

Ethyl alcohol (95%)	—	10 ml.
Formaldehyde	—	6 ml.
Glacial acetic acid	—	6 ml.

Good slides have been obtained without this prefixation but results were more uniformly satisfactory with it.

Various staining schedules were tried with larvae of *Drino bohemica*, which were available in larger numbers. Usually the losses were excessive with systems involving removal of the cover slip. Accordingly, a system was developed for staining and transferring the material to the mounting medium *in toto* before squashing. The schedule used, which follows, is a modification of one outlined by Wilson (11).

1. Place the brain tissue in three drops of iron acetocarmine on a microscope slide for 25 min. (Heating does not make much difference.)
2. Transfer the brain to a drop of 70% ethyl alcohol on another slide.
3. Rinse rapidly on the slide with several quick changes of 70% ethyl alcohol.
4. Rinse rapidly with several quick changes of 95% ethyl alcohol.
5. Rapidly drain off excess alcohol while adding a few drops of a Venetian-turpentine mixture constituted as follows:

Venetian turpentine	25 ml.
Phenol	50 ml.
Propionic acid	35 ml.
Acetic acid	10 ml.
Water	20 ml.

6. Rotate the slide gently but briefly to wash the tissue.
7. Replace the Venetian-turpentine mixture with several new drops, then wipe away all excess around the brain tissue and apply a cover slip immediately.
8. *Gently* squash out the tissue with a smooth, blunt instrument. (Excessive pressure at this stage ruins many preparations. The cover slip must not move laterally during squashing.)
9. Excess Venetian-turpentine mixture can be removed by carefully pressing a paper towel over the cover slip. (It should not take over 1·5 min. from stain to completed preparation.)

Dissecting, staining, and mounting can be completed in less than half an hour and the preparations can be used immediately (with much care when using oil, of course). The preparations should be allowed to stand for several days, during which time the mounting medium dries out slightly under the cover slip and needs replenishing. Some of these slides fade in a few weeks; others develop numerous dark specks of stain that obscure the chromosomes. On the other hand, some of the slides prepared in the summer of 1947 are still usable. The reasons for these variations in behavior are not known. We have tried sealing around the edges of the cover glasses with a thick mixture of clarite in toluol (or thick balsam in xylol) after the initial drying period of a few days. The sealing at least prevents movement of the cover glass when removing immersion oil and may delay deterioration of the preparation.

Analysis

The first step in analysis of the somatic chromosomes of a species is the selection of suitable mitotic figures. Cell size is highly variable, especially in early third instar larvae. A small number of unusually large cells is found at this stage, and fewer still in very early pupae, but in older pupae the cells become more uniformly small. The 12 (in nearly all cases) chromosomes in these large cells separate more readily than in small ones and, since they are longer, the small errors in measurement represent a lower percentage error. Also, the chromosomes in these larger cells stain less strongly; this is sometimes desirable since more details of chromosome structure and morphology can be

seen. On the other hand, these longer chromosomes are more liable to be stretched in the squashing process. Experience shows that the best figures for analysis are those of medium size in which the chromosome pairs are still adjacent to each other, all distinctly separated, and all perfectly flat (Plate II, Fig. 18). Unfortunately such perfect figures are rare even in the best preparations since there are nearly always some small, frustrating imperfections and sooner or later one accepts less perfect ones. To avoid a bias, all figures for analysis were selected before drawing by examination only, and only very rarely was a figure not included for a species after analysis.

One of the chief obstructions to the obtaining of more good figures is the pairing of the homologous chromosomes in these somatic cells. This association is strong through the prophase (so strong that the homologues are often found coiled about each other). As metaphase is achieved the affinity of each chromosome for its homologue decreases somewhat and in squash preparations they are found lying side by side but distinctly separate in many cells. Later, but still before anaphase, they are usually widely separated after squashing. Cells in these stages have been preferred for this study because (*a*) the chromosomes can be measured individually and (*b*) these stages are short so that the chromosomes should be more nearly comparable in a series of figures.

The chromosomes in satisfactory figures were drawn in outline, with a camera lucida, at a magnification of approximately ×3520. In all cases chromosome measurements on these drawings were taken to the nearest 0.05 cm. The total complement length in such drawings ranges from about 14 to 37 cm. with individual chromosomes ranging from about 0.2 to 4 cm. At this magnification the figures can be drawn on 4 × 6-in. index cards and the size of chromosomes in the drawing is very suitable for accurate measurement. A pair of well-made dividers was most useful in the process of actual measurement.

As indicated previously, most mitotic figures are not perfect and accordingly corrections must be made in the measurements of certain chromosomes in nearly all cells. Of course, with a modern research microscope one could calculate rather precisely the exact length of each chromosome by making very careful measurements with an ocular scale supplemented by calculations of changes in the depths of focus. This would be extremely time-consuming and it is very doubtful whether the improvement in results would justify the extra effort. To avoid such drudgery the following arbitrary scale of corrections was used:

(*a*) *Part of a chromosome arm sloping to a different focus.*—If the difference was very slight it was indicated as such on the drawing and, where the corresponding arm of the homologue was longer, 0.05 cm. was added to the measured length of the sloping arm. When the slope was definite but one end of the arm not entirely out of focus when the other was in focus, 0.05 cm. was added. When one end of an arm was definitely out of focus when the other was in focus, 0.10 cm. was added. Greater differences in focus required

additions of 0.15 cm., and in very rare cases more; in still rarer cases the measured length of the corresponding arm of the homologue was substituted. Some consideration of the total arm length must be given in applying these corrections. In the majority of cases less than 6 arms out of 24 required such corrections.

(*b*) *Kinetochores of autosomes.*—The chromosomes seem to be weak at this point and as a result one arm sometimes separates abnormally far from the other arm. In all cases the kinetochore was assigned the value of 0.10 cm. regardless of actual length. This value is very close to the measured distance in the great majority of cases. The suggestion that the kinetochore be omitted in measurements was discarded because it was felt that this region is as much a part of a chromosome as any other. In several cases where the kinetochore was not clearly visible it was assigned the value of 0.10 cm. which was added to the measured length of the autosome.

(*c*) *Main secondary constrictions and kinetochores in sex chromosomes.*—The sex chromosomes are about 0.4 cm. to 0.8 cm. in length, on the drawings, for most species. These short chromosomes are acrocentric in those cases in which the position of the kinetochore has been established. Each usually has a secondary constriction which varies in distinctness in different species and is usually less conspicuous than the kinetochore in the autosomes. It was felt that the use of 0.10 cm. as the standard length of these secondary constrictions would increase the length of the sex chromosomes disproportionately in relation to the other chromosomes. Therefore, 0.05 cm. was used as the standard length and added to the measured length when the secondary constriction could not be seen. In several species the sex chromosomes are acrocentric and comparable to the smaller autosomes in length (more than a centimeter in measured length) and in two they are longer than the autosomes and metacentric. In these species the 0.10 cm. figure was used for the main secondary constrictions and the kinetochores as for the autosomes.

(*d*) *Secondary constrictions.*—These constrictions are at points where distinct achromatic bands extend across the chromosomes (it is not always possible to distinguish them from breaks). They were incorporated into the main arm length, but in those cases where the region exceeded 0.10 cm. its recorded length was limited to 0.10 cm. Main secondary constrictions in sex chromosomes are exceptions as indicated above.

(*e*) *Extraneous chromosome material.*—Since the chromosome number was small (12 in nearly all species), one soon learned to distinguish extraneous chromosome material introduced during squashing. These bodies were drawn in the figures but omitted as measured parts of the complement.

(*f*) *Extra chromosomes.*—Extra chromosomes were found in rare individuals. Their presence was recorded but they were excluded from the analysis.

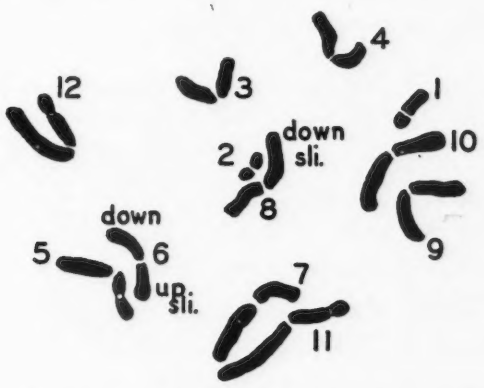
(*g*) *Stretching and compression of chromosome arms.*—Obviously it is very difficult even to estimate the amount a particular arm has been stretched. Where differences in the corresponding arms of homologous partners did not exceed 0.20 cm. they were ignored, except with very short chromosomes.

In several cases an obviously stretched arm was reduced to within 0.1–0.2 cm. of its shorter homologue. In very rare cases where measurement was impossible the length of the apparently normal arm of the homologue was used and this substitution system was extended of necessity to the entire homologous chromosome on very rare occasions. Stretching was most extreme in large figures with long chromosomes. Differences in stretching and compression also exist between different chromosome pairs and constitute an important but unavoidable source of error in squash preparations.

Table II is a summary of the measurements and calculations for the chromosomes illustrated in Text-fig. 1. The results and calculations are largely self-explanatory. All calculations were made with a slide rule and to the first decimal place only, which was considered sufficiently accurate in consideration of errors from preparation and measurement techniques. The arm ratio was calculated by dividing the average length of the two short arms of an homologous pair of chromosomes into the average length of their long arms. The percentage of the total complement length was obtained by dividing the combined lengths of the two homologues of each pair by the total length of the entire complement (TCL) and multiplying the quotient by 100.

This system of calculating pair length as a percentage of the total complement length makes it possible to compare directly complements differing greatly in actual size. This is desirable because of the great variation in complement size in different cells of one individual or of one species and because only a few cells (commonly 12) could be analyzed for each species. Obviously, considerable care is essential when comparing the complements of different species on this basis.

The arm ratio about the kinetochore, calculated as indicated above, has been considered as the primary arm ratio. In acrocentric sex chromosomes



TEXT-FIG. 1. Somatic chromosome complement from a male larva of *Drino bohemica*. ×3520.

it has been considered desirable to calculate an "arm ratio" about the prominent (main) secondary constriction that is usually present and easily recognizable because of the distinct achromatic band across the chromosome. These "arm ratios" will be referred to as secondary arm ratios and were calculated by dividing the larger segment (in relation to the main secondary constriction) by the smaller. Where the sex chromosomes are not definitely known to be acrocentric the term arm ratio will be used.

The chromosome pairs were numbered I–VI in the order of increasing size on the basis of average lengths of the homologous pairs except that the *X*-chromosomes (see below) are always Pair I regardless of their relative lengths. Species with several pairs of chromosomes of nearly the same lengths gave most

TABLE II

Drino bohemica,—ANALYSIS OF THE CHROMOSOME COMPLEMENT SHOWN IN TEXT-FIG. 1

Chromosome No.	Chromosome measurements in cm. on the drawing		Length of pair in cm.	Percentage of TCL†	Arm ratio, long : short
1.	0.45 + 0.05 + 0.25	= 0.75 × 2	I=1.50	7.7	1.80††
2.	0.25 + 0.05 + 0.20	= 0.50		(5.1)	(1.25)
3.	0.70 + 0.10 + 0.60	= 1.40	II=2.80	14.3	1.17
4.	0.70 + 0.10 + 0.60	= 1.40			
5.	0.90 + 0.10 + (0.30 + 0.05 + 0.35)** + 0.10* + 0.05	= 1.70	III=3.25	16.6	1.26
6.	0.70 + 0.10 + 0.60	= 1.55			
7.	1.00 + 0.10 + 0.75 + 0.05	= 1.85	IV=3.60	18.4	1.34
8.	0.90 + 0.10 + 0.70	= 1.75			
9.	0.85 + 0.10 + 0.85	= 1.80	V=3.70	18.9	1.06
10.	0.95 + 0.10 + 0.85	= 1.90			
11.	1.35 + 0.10 + 0.95	= 2.40	VI=4.75	24.2	1.40
12.	1.30 + 0.10 + 0.95	= 2.35			
Total complement length (TCL)†			19.60	100.1	Av., II–VI 1.25

* *The superscript figures are for corrections as recorded on the drawing.*

** *The short arm of chromosome five had a medial secondary constriction.*

† *The percentage of total complement length (TCL) for the Y-chromosome (No. 2) is calculated from the figure for the X-chromosome (No. 1) and is not included as part of the total complement length.*

†† *The "arm ratio" for the acrocentric X-chromosome (No. 1) refers to a secondary constriction, i.e., this is a segment ratio rather than a true arm ratio. This holds for most other species in the Tachinidae. It holds also for the Y-chromosomes.*

difficulty in distinguishing pairs because sometimes shorter pairs stretched more than longer pairs. However, the arm ratios, positions of secondary constrictions, and other morphological characters were valuable secondary means of identifying chromosomes in practically all species and were used as criteria where length alone left doubt in assigning the proper pair number to a chromosome. Naturally, these difficulties decreased with increasing familiarity with the complements of particular species.

Another complication in these analyses arises from the heteromorphic nature of the sex chromosomes. Some individuals have two apparently identical homologues as Pair I, whereas other individuals have two different chromosomes. The logical interpretation seems to be to regard individuals possessing a heteromorphic pair as males, the usual situation in the Diptera. Accordingly, the chromosome type found to be represented twice in certain larvae and once in others is referred to as the *X*-chromosome (or I) and its heteromorphic homologue as the *Y*-chromosome, subject to later revision if necessary. In calculating percentages of total complement length for individuals carrying the heteromorphic pair (presumably males), double the length of the *X*-chromosome is substituted for the sum of the lengths of the heteromorphic partners so that all calculations for males and females are on a comparable basis. The term *total complement length*, or TCL, wherever used in this report thus represents, for females, the true total complement length and for males the corrected total complement length.

Since it seemed possible that the true total complement length for females might differ from the corrected total complement length for males, results from six cells each from a male and a female larva were compared (Table III). The results were so similar that it seemed reasonable to accept the corrected total complement length for males as equivalent to the total complement length of female larvae.

TABLE III

Drino bohemica,—SOMATIC CHROMOSOME COMPLEMENTS OF A MALE AND A FEMALE

Pair number	Average arm ratios		Average percentages of TCL		Av. lengths, μ	
	♂	♀	♂	♀	♂	♀
I	2.34	2.26	6.9	7.3	2.4	2.6
II	1.12	1.14	15.0	14.4	5.2	5.3
III	1.12	1.13	16.3	16.4	5.7	6.1
IV	1.27	1.34	17.4	17.8	6.0	6.6
V	1.14	1.20	19.2	19.2	6.7	7.1
VI	1.35	1.41	25.3	24.8	8.8	9.1
Av.	1.39	1.41	16.7	16.6	5.8	6.1

Comparison of the somatic complements of different species revealed another problem. Can one be sure that a chromosome pair of one species is homologous to a particular chromosome pair of another species? One very definitely cannot be sure of such homology. Only studies of meiosis or salivary chromosomes in interspecific hybrids could establish such homology without question. But since the primary objective in this study at present is the comparison of species complements it is not essential that homologous chromosome pairs of different species be compared directly as such. Accordingly, for example, Pair III of one species is not necessarily homologous to Pair III of another species.

In the idiograms (Text-figs. 3 to 14) the percentages of TCL are shown as the ordinate and the chromosome pairs along the abscissa. The *Y* has been included in its proper percentage relation to the *X* (Pair I). The *Y*- and *X*-chromosomes are followed by the autosomes in order of increasing size. Kinetochores are shown as V-shaped indentations on both sides of the chromosomes; thus when the kinetochore is at or near the end, the chromosome is pointed. Important secondary constrictions are indicated by narrow white bands across the chromosome at the proper position. Less distinct secondary constrictions, not regularly found, are indicated by an arrow on one side of the chromosome at approximately the usual position. In comparing these idiograms of complements it is very important to keep in mind that (1) Pairs I–VI always total 100% of the TCL and (2) a particularly large chromosome, such as the *X* in *Winthemia datanae*, causes a decrease of *all* the other pairs in percentage of TCL, so that the autosomes look much smaller in this species than in others, though this is not necessarily so in actual measured length.

The term heterochromatic is used to designate chromosomes, or parts of them, which stain more strongly (positively heterochromatic) or less strongly (negatively heterochromatic) than usual at metaphase. Heteropycnotic is applied to chromosomes, or parts of them, which remain dense and stain deeply in otherwise reticulated metabolic nuclei.

The photomicrographs were made with a ×60 oil immersion apochromatic objective with ×20 ocular. Eastman Contrast Process Panchromatic film developed in D11 developer gave the best results. Nearly all prints were made to give a magnification of approximately ×2250 and reduced to ×1800 in publication.

Descriptions of Somatic Complements of Tachinid Species

The somatic chromosome complements of 16 species of the Tachinidae have now been examined. For 11 of these, at least 12 complements representing several larvae have been analyzed; for the remaining five, observations were less extensive. Detailed descriptions of the chromosome complements analyzed are given below, whereas comparisons of the complements are made in a later section of this paper.

Aplomya caesar (Ald.)

Two collections of this species were studied.

The first collection of larvae was from European corn borer, *Pyrausta nubilalis* larvae obtained in Ontario. Twelve chromosomes were found in

each of the 12 cells analyzed from a total of seven larvae, four males and three females. The complements ranged in total length (TCL) from 40.3 μ to 74.4 μ and averaged 57.7 μ. The male complement is shown in Plate I, Fig. 2, and the results of analysis in Table IV and Text-fig. 3.

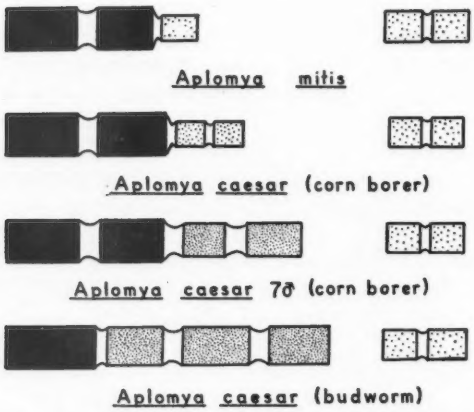
TABLE IV

Aplomya caesar,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(X)	II	III	IV	V	VI	I(X)	II	III	IV	V	VI	
1♂	2.14	1.71	1.50	1.58	2.24	1.38	11.7	14.8	15.5	16.5	17.5	24.0	58.6
2♀	—	1.54	2.00	1.80	1.97	1.52	11.6	15.0	15.2	17.2	17.6	23.3	70.2
3♂	—	1.82	1.54	1.16	2.23	1.63	12.2	14.9	15.9	16.5	17.0	23.5	62.9
3♂	1.44	1.54	1.32	1.52	2.29	1.31	11.1	14.0	14.9	17.4	20.0	22.6	59.1
3♂	—	1.33	1.55	1.76	1.86	1.47	11.1	14.0	15.8	16.4	16.9	25.8	53.9
3♂	1.71	1.32	1.84	1.31	1.65	1.42	11.6	15.1	16.0	17.7	17.9	21.7	51.7
4♂	—	1.45	1.67	1.46	2.42	1.37	9.2	15.4	16.0	16.2	19.7	23.5	74.4
4♂	1.50	1.50	1.32	1.73	1.75	1.52	9.1	14.4	16.5	17.0	18.6	24.4	53.5
5♀	1.83	1.75	1.52	2.14	2.35	1.69	9.5	14.1	15.9	17.3	19.2	24.0	59.9
5♀	1.69	1.62	2.00	2.16	2.19	1.30	11.5	15.0	15.5	15.5	18.1	24.4	55.9
6♀	1.43	1.53	1.43	2.15	2.46	1.62	13.1	14.8	15.2	15.9	17.3	23.6	40.3
7♂	—	1.38	1.89	1.76	2.30	1.72	14.1	14.1	15.3	16.1	18.2	22.3	52.0
Average	1.68	1.54	1.63	1.71	2.14	1.50	11.3	14.6	15.6	16.6	18.2	23.6	57.7

Pair I is distinct in size, Pairs II, III, and IV overlap appreciably in arm ratio and cannot be distinguished regularly on the basis of relative length alone. Lengths of II and III overlap only slightly. Some errors must occur in the separation of III and IV. Pairs IV and V differ strongly in ratio and by 2% in length, whereas Pairs V and VI, which also differ strongly in ratio, have a 5.2% difference in length. Hence IV and V, and V and VI, are separable in nearly all cases. Arm ratios for Pairs II–VI average 1.70 placing this species in what is designated as the high arm-ratio group.

The *X*-chromosomes in this species are noticeably variable in size and apparently also in morphology (Text-fig. 2 and Plate I, Figs. 2 and 3). In this first collection each consists of four parts separated by secondary constrictions, two larger denser segments at one end, and two small dots at the other. The constriction between the two large segments has been used as the main secondary constriction for calculating the secondary arm ratios. These ratios varied considerably (note particularly the *X*-chromosome in 7♂, Table IV, shown in Text-fig. 2) so that the collection may represent a complex of different types.



TEXT-FIG. 2. Sex chromosomes of *Aplomya* species. The *X*-chromosomes on the left and *Y*-chromosomes on the right. Black parts stain like the autosomes; stippled parts vary in density of stain as indicated.

Secondary constrictions have been noticed in several different pairs. Pair II has a noticeable medial constriction in the short arm in five of the 12 figures and Pair VI has a similarly located constriction regularly observable in the short arm. Pair V has a constriction in the long arm fairly close to the kinetochore. It is probably this constriction that is sometimes seen rather than the primary one (kinetochore), and as a result the arm ratio is reduced by about 0.5 or more.

The *Y*-chromosome averaged 31% of the *X*-chromosome for the eight cells where it was present, and had an arm ratio of about 1.00 in two of them. Its total length corresponds to only 3.5% of the TCL and since it stains lightly it is often hard to see clearly (as in *Aplomya mitis*). The parts of the *X* adjacent to what has been assumed to be the main secondary constriction stain more darkly at metaphase and may be the small heteropycnotic regions sometimes noticeable in metabolic nuclei.

A single larva from the second collection, on the spruce budworm, *Choristoneura fumiferana*, was studied. Twelve chromosomes were present in each of the six somatic complements drawn and analyzed. The complements ranged from 52.7 μ to 65.5 μ with an average of 55.9 μ in total length. The average results of analysis of the six complements were:

	I	II	III	IV	V	VI
Arm ratio	2.23*	1.45	1.51	1.74	1.97	1.53
Percentage of TCL	15.4	13.8	14.9	15.6	17.5	22.8

* *Secondary arm ratio.*

The secondary arm ratio for Pair I was calculated as for the previous collection but in this case only one large, denser segment was found at one end (Text-fig. 2 and Plate I, Fig. 3) and the secondary constriction adjacent to it was usually less conspicuous than the other two. However, calculating the arm ratio about this less conspicuous constriction gives a result more nearly comparable to that of the previous collection. The central constriction may be primary, since no anaphases were seen by which this point could be decided.

The results for this single larva from the budworm are very interesting. The *X*-chromosomes (Pair I) differ from the usual type found in the collection from the corn borer both in size (15.4% vs. 11.3%) and morphology (Text-fig. 2). One larva from the corn borer (7 ♂) did have an *X*-chromosome resembling that found in the one from the budworm. In other respects all of these larvae, from both collections, have very comparable chromosomes. The single larva from the budworm apparently represents either extreme variation in type of *X*-chromosome *or* a distinct subgroup of this species attacking the budworm. Unfortunately, a decision regarding these alternatives must await further investigation.

Aplomya mitis (Meig.)

Larvae of this species were obtained from budworms, *Cacoecia rosana*, imported from Europe. Twelve chromosomes were found in all 12 cells analyzed from four male and one female third instar larvae. The complements ranged from 40.4 μ to 55.9 μ with an average of 45.5 μ in total length. Plate I, Fig. 4, is an illustration of the complement of a male and the results of analysis are summarized in Table V and Text-fig. 4.

TABLE V

Aplomya mitis,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1♂	1.43	1.47	1.45	2.16	2.46	1.32	10.4	14.0	14.5	16.7	21.1	23.3	52.0
2♂	1.80	1.53	1.35	2.06	2.00	1.52	10.1	14.9	16.1	17.7	18.3	22.8	45.0
2♂	—	1.27	1.50	1.83	2.12	1.46	8.2	13.8	16.3	17.6	20.1	24.1	55.9
3♀	—	1.65	1.78	1.89	2.28	1.55	8.2	14.8	16.3	18.1	19.0	23.6	47.1
3♀	—	1.24	1.47	1.71	2.00	1.60	9.7	14.2	15.9	17.3	19.0	23.8	41.3
3♀	—	1.39	1.35	1.95	2.10	1.45	9.5	14.0	15.2	18.7	18.7	23.8	47.8
3♀	1.40	1.44	1.33	1.87	2.00	1.39	9.0	14.9	16.0	17.4	18.1	24.6	41.0
4♂	1.50	1.25	1.47	1.81	2.37	1.56	8.3	13.9	16.0	17.0	20.1	24.6	41.0
4♂	1.65	1.47	1.53	1.87	2.25	1.46	9.2	14.4	16.6	17.6	19.7	22.5	40.4
4♂	1.50	1.22	1.61	1.94	2.63	1.66	8.9	14.0	16.2	17.8	19.8	23.2	44.7
5♂	1.33	1.42	1.75	2.07	2.42	1.62	9.0	13.9	15.5	16.1	19.7	25.8	44.1
5♂	—	1.44	1.45	2.12	2.32	1.58	8.1	14.9	16.5	16.8	17.7	26.0	45.8
Average	1.51	1.40	1.50	1.94	2.25	1.51	9.1	14.3	15.9	17.4	19.3	24.0	45.5

Pair I chromosomes are shorter than in *Aplomya caesar*. There is some overlapping in the arm ratios for Pairs II and III but since they differ in length (14.3% vs. 15.9%) no appreciable difficulty was encountered in distinguishing them. Other pairs differ appreciably in arm ratios, and overlap little in percentages of TCL; hence recognition of pairs is moderately certain. The average of arm ratios for Pairs II–VI was 1.72 for the figures analyzed, which would place this species in the high arm-ratio category.

In two cells a medial secondary constriction was found in the short arm of Chromosome VI. The terminal portion of the longer "arm" of the *X*-chromosome stains more lightly than the proximal segment. Perhaps secondary constrictions were less apparent in this species because the complements studied were generally shorter (and possibly more contracted) than in most other species analyzed.

The *Y*-chromosome is very small (44.6% of the small *X* and corresponds to 4.0% of the TCL) and since it stains lightly it is difficult to determine its arm ratio. It is barely distinguishable as a small dark spot near the *X* in Plate I, Fig. 4 (note the remains of the nucleolus also present at the bottom of this cell). Small heteropycnotic regions probably represent the sex chromosomes in some metabolic nuclei but no definite proof that they involve the *X*- or *Y*-chromosomes was obtained.

Ceracia dentata (Coq.)

A single third instar larva of this species was obtained from a grasshopper, *Melanoplus* sp. in late July of 1951 at Belleville. It had 12 chromosomes and was apparently a male (Plate I, Fig. 5). Results of analyses of four complements are shown in Table VI and Text-fig. 5.

The percentage values are less useful in this species than in others. In particular, Pairs II, III, and IV differ slightly in length and V is only slightly

TABLE VI

Ceracia dentata,—ANALYSIS OF FOUR CELLS FROM A MALE LARVA

Chromosome pair	Long : short arm ratios					Percentages of TCL				
	1	2	3	4	Av.	1	2	3	4	Av.
I(*X*?)*	—	2.22	3.00	—	2.61	7.8	7.0	5.0	4.8	6.2
II	1.09	1.06	1.13	1.07	1.09	15.7	16.9	17.6	17.0	16.8
III	1.87	1.67	1.54	1.58	1.67	16.3	16.9	17.6	17.6	17.1
IV	1.22	1.03	1.43	1.25	1.23	17.9	17.7	18.1	17.8	17.9
V	2.18	2.40	2.55	2.40	2.38	18.9	18.0	18.7	19.2	18.7
VI	1.34	1.31	1.23	1.18	1.27	23.5	23.5	23.0	23.6	23.4
Av., II–VI	1.54	1.49	1.58	1.50	1.53					
TCL, μ						43.7	57.1	56.5	53.5	52.7

* *Corresponding percentages for the Y?-chromosomes and their arm ratios (in parentheses) are 7.1 (1.25), 7.0 (1.17), 4.5 (?), 4.3 (1.33), giving averages 5.6 for percentage TCL and 1.25 for arm ratios in the four cells.*

longer than IV. However, the arm ratios of Pairs III and V are very distinct from those of Pairs II, IV, and VI. Accordingly, the pairs can be separated with reasonable certainty.

Since the figures analyzed were small it is not surprising that no secondary constrictions, except those in the *X*- and *Y*-chromosomes, were seen. These sex chromosomes have only been seen in one individual and it is, of course, impossible to say which is which. In Text-fig. 5 the shorter one has been arbitrarily designated as the *Y*-chromosome. They are not very heterochromatic as seen at metaphase but are recognizable in metabolic nuclei and are accordingly heteropycnotic. Although they are similar in size and acrocentric, their arm ratios about the secondary constriction are decidedly different.

Ceromasia auricaudata Tns.

The larvae of this species were reared in laboratory stocks of the spruce budworm, *Choristoneura fumiferana*, collected in British Columbia. Cells from two males (one an early pupa) and five females were analyzed and possessed 12 chromosomes as their somatic complements. The complements ranged from 42.3 μ to 62.6 μ and averaged 54.8 μ in total length. The results of analyses are shown in Table VII and in Text-fig. 6. The chromosomes are illustrated in Plate I, Fig. 6.

The figures were not difficult to analyze and resemble those of *Drino bohemica* in most respects. Pair I is distinct, being small in size (6% of the TCL) and acrocentric. The differences between the other pairs in percentage of TCL were sufficient to make pair identification satisfactory in most

TABLE VII

Ceromasia auricaudata,—ANALYSIS OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1♀	1.62	1.30	1.12	1.32	1.11	1.23	6.5	16.1	16.6	17.5	17.7	25.6	50.5
1♀	1.60	1.33	1.24	1.24	1.17	1.29	5.9	16.8	17.7	18.2	18.7	22.7	62.6
2♀	—	1.15	1.30	1.43	1.12	1.20	6.7	15.0	16.0	17.6	18.2	26.5	44.6
2♀	1.41	1.27	1.21	1.07	1.21	1.29	5.5	15.7	16.6	18.6	19.8	23.8	49.0
2♀	2.00	1.19	1.39	1.39	1.07	1.11	5.4	15.4	16.7	18.0	18.0	26.6	76.7
2♀	—	1.13	1.37	1.50	1.35	1.08	5.3	16.1	17.8	18.7	19.0	23.1	48.7
3♂	2.00	1.15	1.13	1.27	1.05	1.22	5.0	15.0	17.0	17.5	19.5	26.0	56.9
3♂	—	1.15	1.18	1.18	1.08	1.24	7.4	15.9	17.6	17.6	18.3	23.3	42.3
4♂	1.40	1.08	1.28	1.26	1.10	1.32	7.0	15.0	16.3	17.4	17.9	26.5	53.2
4♂	—	1.15	1.10	1.33	1.12	1.49	6.1	15.2	16.9	16.9	18.2	26.7	56.4
5♂	—	1.10	1.12	1.52	1.06	1.40	5.4	16.0	17.8	17.8	18.3	24.7	57.2
5♂	—	1.36	1.16	1.38	1.12	1.34	5.7	16.7	16.9	17.4	17.9	25.5	59.8
Average	1.67	1.20	1.22	1.32	1.13	1.27	6.0	15.7	17.0	17.8	18.5	25.1	54.8

cases. This was fortunate since the differences between the arm ratios of these pairs are small (range 1.13 to 1.32) and accordingly not very helpful. The low arm-ratio values for Pairs II–VI (av. 1.23) place this species in the low arm-ratio group.

Secondary constrictions are distinct in Chromosome VI. The long arm has a proximal segment averaging 1.95 times the distal portion for nine chromosomes. The shorter arm has a secondary constriction that is nearly medial so that the proximal portion averaged 0.96 of the distal in six chromosomes. Secondary constrictions occasionally noticed in the other pairs were not regularly observed in any case and accordingly are of doubtful value as distinguishing characters of the chromosomes of this species.

The *Y*-chromosomes averaged 71.6% of the *X* in length, corresponded to 4.4% of the TCL, and were less chromatic than the parts of the *X* that did not differ noticeably in staining reaction from the autosomes. Three of them averaged 1.22 in arm ratio. In two larvae, two adjacent, very tiny, slightly chromatic rods, which apparently constitute a single chromosome, were noticed in addition to the six pairs. There was some positive heteropycnosis of the *X*-chromosomes, but they are so small that they are inconspicuous in metabolic nuclei.

The analyses mentioned above were completed in 1949. In 1951 two other larvae were obtained in the same host from British Columbia. One had 12 chromosomes, including what appeared to be a small heteromorphic pair of acrocentric chromosomes; the other had 13 chromosomes, including what appeared to be the same small heteromorphic pair plus a tiny chromosome consisting of two tiny dots. A complement containing the extra chromosome is shown in Plate I, Fig. 7. The average results of analyses of eight cells from these two apparently male larvae are shown below:

	(*Y*)	I	II	III	IV	V	VI
Arm ratio	1.63	2.49	1.16	1.23	1.19	1.21	1.30
Percentage of TCL	4.0	5.4	15.7	17.0	17.6	18.5	26.0

These results agree well with the earlier ones except for Pairs I and VI. The difference in both length and secondary arm ratio for both the *X* and *Y* are unusually high. Of course they are very small, which leads to greater errors, but in this species their shape seemed to be more strongly affected by squashing than in most species. In these larvae the *X* and *Y* stained more lightly at metaphase than in the previous collection. The differences may have resulted from the elimination of the ethyl alcohols in preparation of these newer slides. This species may, however, be more variable than others.

The tiny extra chromosomes in this species are interesting. Such a chromosome has been found in a larva considered to be *XX* and in one considered to be *XY*. They appear to be supernumeraries.

Drino bohemica Mesn.

Larvae of this species (formerly referred to as *Sturmia* sp.) were available in large numbers from stocks reared in the laboratory on the red-headed pine sawfly, *Neodiprion lecontei*, and accordingly many complements of different individuals were studied. In all cases 12 chromosomes were found in each somatic complement. A complement from a male is shown in Text-fig. 1 and a comparison of averages of six male complements with six female complements in Table III. The 27 somatic complements (from 6 males and 10 females including those in Table III), results for which are recorded in Table VIII and in Text-fig. 7, averaged 63.7 μ in total length with a range from 45.3 μ to 88.7 μ. A photomicrograph of the female complement is shown in Fig. 8 of Plate I.

In the arm ratios shown in Table VIII the average for Pair I is 1.82 as compared with 2.34 and 2.26 in Table III. It is possible that two distinct

TABLE VIII

Drino bohemica,—ANALYSES OF SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(X)	II	III	IV	V	VI	I(X)	II	III	IV	V	VI	
1♀	—	1.08	1.07	1.45	1.07	1.71	6.8	15.3	17.8	16.4	19.5	24.1	50.5
2♀	—	1.40	1.06	1.29	1.09	1.50	8.8	13.6	16.7	17.1	20.2	23.5	64.8
3♀	—	1.23	1.34	1.29	1.10	1.32	7.5	14.1	16.4	18.7	19.1	24.2	62.5
4♂	—	1.07	1.11	1.17	1.09	1.33	7.5	13.4	16.3	17.2	20.9	24.7	68.0
5♀	1.80	1.07	—	1.57	1.10	1.48	7.1	13.7	17.7	16.8	19.4	25.3	64.5
6♂	—	1.17	1.19	1.42	1.13	1.30	7.2	14.2	17.6	17.8	18.9	24.2	67.0
7♀	1.80	1.14	1.13	1.29	1.13	1.37	7.1	14.6	16.0	17.1	19.6	25.7	61.1
8♀	1.80	1.21	1.13	1.30	1.18	1.36	7.3	15.2	16.6	17.3	18.0	25.6	60.1
9♀	—	1.09	1.05	1.40	1.19	1.31	8.2	15.1	16.8	16.4	18.3	25.1	45.3
10♀	1.80	1.08	1.12	1.32	1.10	1.35	8.4	15.2	16.0	17.4	18.8	24.2	50.7
11♂	—	1.08	1.07	1.26	1.17	1.41	8.1	15.1	16.2	17.5	18.1	25.0	52.8
11♂	1.80	1.08	1.23	1.32	1.09	1.36	7.8	15.1	16.1	18.0	18.0	25.0	54.7
12♀	1.80	1.21	1.07	1.06	1.06	1.37	8.5	15.2	17.0	17.5	18.6	23.1	53.5
13♂	1.90	1.08	1.18	1.37	1.13	1.40	7.1	15.0	16.9	17.7	18.2	25.1	54.8
14♂	1.85	1.09	1.17	1.36	1.42	1.33	6.8	13.9	16.2	18.2	19.4	25.5	49.2
Average	1.82	1.14	1.14	1.32	1.14	1.39	7.6	14.6	16.7	17.4	19.0	24.7	57.3
Average of 27 cells*	2.09	1.14	1.13	1.32	1.16	1.39	7.4	14.7	16.5	17.5	19.1	24.8	63.7

* *Results for 12 complements from Table III are included.*

subgroups are represented. The kinetochores of Pairs II, III, and V are more nearly medial than those of Pairs IV and VI. Except for III and IV the pairs are usually distinct in length and they can be separated, as a rule, on the basis of this difference. In general, the average ratios for Pairs II–VI are low and the species accordingly belongs to the low arm-ratio group.

Secondary constrictions are rarely observable in the chromosomes of this species. In some cells the shorter arm of Chromosome VI has a constriction in the distal half, about 0.66% to 0.75% of the arm length from the kinetochore.

The *Y*-chromosome averaged 51% of the *X*, and corresponded to 3.8% of the TCL, in 12 cells. The average arm ratio for eight cells, where the arms were distinguishable, was 1.21. The *X*-chromosome stains normally at metaphase but the *Y*-chromosome is usually weakly stained though, of course, this appearance may be due partly to the small size of its segments.

Eumea westermanni (Zett.)

Several larvae of this species were obtained from *Cacoecia rosana* collected in Europe. The chromosomes were analyzable in only one cell from a female (probably) and the results are only included because of the rarity of this species. There were clearly 12 chromosomes present. It was assumed that members of the small pair were the *X*-chromosomes. The results of the analysis of this single cell are shown below:

	I	II	III	IV	V	VI
Arm ratio	2.17	1.95	1.03	1.13	1.13	1.12
Percentage of TCL	5.9	16.3	17.4	19.1	20.1	21.3

The total complement length was 50.8 μ in this cell and the average ratio for Pairs II–VI was 1.28, which indicates a low arm-ratio category for this species. The *X*-chromosomes are noticeably small and not unusually dark in comparison with the autosomes. Secondary constrictions were present in Pair III on the short arm near the primary constriction.

Lydella grisescens R.D.

A third instar larva, apparently a male, was taken from the European corn borer, *Pyrausta nubilalis*, collected in southern Ontario. The preparation of the chromosomes for study was not good, but four somatic chromosome complements were drawn and analyzed. Each of the four consisted of 12 metacentric chromosomes and the complements ranged in length from 58.4 μ to 77.3 μ with an average of 69.8 μ. The results of analysis of the complements are shown in Table IX.

TABLE IX

Lydella grisescens,—ANALYSIS OF FOUR CELLS FROM A MALE LARVA

Chromosome pair	Long: short arm ratios					Percentages of TCL				
	1	2	3	4	Av.	1	2	3	4	Av.
(*Y*?)	1.45	1.06	1.08	1.39	1.24	(19.8)	(16.5)	(19.8)	(17.6)	(18.4)
I(*X*?)	1.23	1.33	1.00	1.45	1.25	20.6	18.0	20.5	19.9	19.8
II	1.17	1.24	1.17	1.23	1.20	13.5	14.6	14.7	13.9	14.2
III	1.06	1.07	1.11	1.24	1.12	13.7	15.1	15.0	15.2	14.8
IV	1.29	1.13	1.11	1.14	1.17	15.2	16.6	15.6	15.8	15.8
V	1.08	1.36	1.24	1.23	1.23	16.8	17.1	16.3	16.0	16.5
VI	1.40	1.18	1.47	1.35	1.35	20.2	18.6	18.0	19.1	19.0
Av., II–VI	1.20	1.18	1.22	1.24	1.22					
TCL, μ						70.4	58.4	77.3	72.9	69.8

Pairs II and III do not differ much in length and the same applies to Pairs IV and V. Pair III has a lower arm ratio than Pair II in most cases. Pair IV also has a low arm ratio but the average for Pair V is only slightly higher. Accordingly it is difficult to recognize the pairs. It is clear, however, that this species belongs in the low arm-ratio group.

Since the complements analyzed came from a single larva the *X*- and *Y*-chromosomes cannot be identified. Both are metacentric, are positively heterochromatic at metaphase, and appear as elongate chromatic bodies in metabolic nuclei. At metaphase, the one arbitrarily designated as *X* (Pair 1) is, on the average, slightly longer than Chromosome VI but of course more densely stained. The *Y* (presumably) is slightly smaller and has a subterminal constriction in the longer arm.

The longer arms of the large Pair VI each have two secondary constrictions, one of which is subterminal and the other about a third of the arm length from the primary constriction. Other secondary constrictions were noticed but not consistently at the same location.

Madremyia saundersii (Will.)

The third instar larvae studied were taken from spruce budworm larvae. The 12 cells analyzed came from three males and four females and each contained 12 chromosomes. The range in total complement length was from 41.3 μ to 79.3 μ and the average 52.8 μ. The complement of a female is shown in Plate I, Fig. 9, and the results of the analysis in Table X and Text-fig. 8.

As seen in Table X there is less than the usual range of difference in percentage of total complement length between the longest and shortest chromosome pairs in this species. Since this range (12.9% to 20.5%) is so low it is

TABLE X

Madremyia saundersii,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)*	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1♂	1.33	1.27	1.12	1.39	1.33	1.10	12.9	15.7	16.6	17.2	17.5	20.1	48.8
1♂	1.37	1.25	1.19	1.18	1.21	1.12	11.5	15.9	16.8	17.9	18.1	19.8	51.8
2♂	1.50	1.21	1.18	1.22	1.20	1.14	13.7	14.3	16.2	17.1	18.3	20.5	45.8
2♂	1.13	1.08	1.27	1.33	1.13	1.10	12.9	14.9	15.8	17.4	19.0	20.0	79.3
2♂	1.09	1.19	1.27	1.14	1.21	1.17	13.0	13.5	16.8	17.3	18.4	21.0	52.7
3♀	1.33	1.47	1.35	1.24	1.17	1.08	12.1	14.1	17.6	17.6	18.6	20.0	41.3
4♀	1.69	1.29	1.32	1.16	1.32	1.12	12.9	14.9	15.8	16.7	17.8	21.8	49.5
4♀	1.65	1.17	1.30	1.08	1.38	1.06	13.1	15.6	15.9	16.2	18.4	20.7	51.0
4♀	1.25	1.00	1.25	1.29	1.20	1.07	13.6	15.7	16.8	17.1	17.7	19.1	49.1
5♀	1.33	1.30	1.40	1.50	1.19	1.07	13.5	15.3	15.9	16.5	18.6	20.2	46.5
6♀	2.23	1.18	1.26	1.12	1.21	1.21	12.4	14.6	15.5	16.6	18.6	22.2	50.5
7♂	1.21	1.24	1.16	1.52	1.31	1.09	13.6	14.7	15.5	16.3	19.5	20.4	67.0
Average	1.43*	1.22	1.26	1.26	1.24	1.11	12.9	14.9	16.3	17.0	18.4	20.5	52.8

* *Secondary arm ratios.*

not surprising to find overlapping in the ranges of lengths of pairs such as III (15.5%–17.6%) and IV (16.2%–17.9%). This makes the assignment of pair numbers difficult, and, since the arm ratios for Pairs II to V do not differ significantly, errors in assignment must surely occur. These errors should not affect the arm-ratio average for Pairs II to VI (1.22), which remains as a valid basis for placing this species in the low arm-ratio category.

In several cells a secondary constriction was noticed near the distal end of the short arm of Chromosome VI. This constriction was not observed when the chromosome was in a more compact condition.

The *Y*-chromosome averaged 32.8% of *X* (corresponding to 4.3% of the TCL) in six cells, and in the only case measurable had a secondary arm ratio of 1.00. The *X*-chromosome does not stain excessively at metaphase and heteropycnosis, if present, is not obvious.

Mericia ampelus (Wlk.)

Only two cells from a third instar larva of this species were studied. The presence of a small heteromorphic pair of chromosomes indicates it was a male and both cells contained a complement of 12 chromosomes. The complements were small: 43.8 μ and 41.6 μ. In Table XI there is a summary of the results of analysis and in Plate I, Fig. 10, a photomicrograph of the complement.

TABLE XI

Mericia ampelus,—ANALYSES OF SOMATIC CHROMOSOMES IN ONE LARVA

Chromosome pair	Long: short arm ratios			Percentages of TCL		
	1	2	Av.	1	2	Av.
Y(?)	—	—	—	(3.8)	(3.4)	(3.6)
I(*X*?)	1.00	1.25	1.12	7.1	6.9	7.0
II	2.00	1.67	1.84	15.9	15.1	15.5
III	1.47	1.25	1.36	16.6	16.8	16.7
IV	1.21	1.38	1.30	18.5	18.8	18.6
V	1.33	1.08	1.20	19.5	19.9	19.7
VI	1.03	1.07	1.05	22.4	22.6	22.5
Av., II–VI	1.41	1.29	1.35			
TCL, μ				43.8	41.6	42.7

The number of cells examined is insufficient to permit extensive consideration of the results. It is clear, however, that the *X* and *Y* are very small and it appears that the species belongs in the low arm-ratio group. The *X*- and *Y*-chromosomes were not noticeably heterochromatic nor was heteropycnosis noticed in metabolic nuclei. No secondary constrictions were seen.

Nemorilla pyste (Wlk.)

The two third-instar larvae of this species were from laboratory-reared stock collected in British Columbia on the spruce budworm. Both had 12 chromosomes as their somatic complements and were assumed to be females since no heteromorphic pair of chromosomes was found. Two of the complements analyzed each had a total length of 61.9 μ and the other totalled 43.1 μ. In Table XII is a summary of the results of analyses of the complements from the three cells suitable for study.

TABLE XII

Nemorilla pyste,—ANALYSES OF THREE SOMATIC COMPLEMENTS IN TWO LARVAE

Chromosome	Long: short arm ratios				Percentages of TCL			
	1	2	3	Av.	1	2	3	Av.
I (*X*?)	1.15	1.11	1.27	1.18	13.5	13.5	12.0	13.0
II	1.29	1.41	1.33	1.34	15.6	14.9	15.4	15.3
III	1.06	1.09	1.06	1.07	15.6	15.9	15.6	15.7
IV	1.41	1.47	1.23	1.37	17.0	16.5	16.3	16.6
V	1.25	1.17	1.14	1.19	17.5	18.5	19.1	18.4
VI	1.05	1.11	1.14	1.10	20.7	20.7	21.6	21.0
Av., II–VI	1.21	1.25	1.18	1.21				
TCL, μ					61.9	43.1	61.9	55.4

Pair I may be the *X*-pair since these chromosomes were slightly heterochromatic in one cell. Except for II and III, the pairs are distinct in percentage of TCL. Pairs II and III differ in arm ratio; II averaged 1.34 and III, 1.07. Also, Pair IV is appreciably higher in arm ratio then III or V. Apparently this species belongs to the low arm-ratio group since the average of arm ratios for Pairs II–VI is 1.21 (if the average of three complements is acceptable).

A subterminal secondary constriction was found on the distal portion of the short arm of Chromosome VI. Heteropycnosis was not observed in metabolic nuclei.

Neophorocera hamata (A. and W.)

The third instar larvae studied were parasites of the red-headed pine sawfly, *Neodiprion lecontei*, and were reared under controlled temperatures. Each of the 12 cells analyzed, from one male and four females, contained 12 chromosomes. The complements were unusually long, averaging 68.3 μ with a range from 56.2 μ to 93.1 μ in TCL. The results of analysis are shown in Table XIII and Text-fig. 9. The chromosomes of a female complement are illustrated in Plate I, Fig. 11.

The *X*-chromosome is the shortest found in any of the species studied so far. The identification of other pairs is moderately satisfactory on the whole but not certain in all cases. For example, Pairs III and IV differ by only 0.9% in average length and only 0.07 in arm ratio and, since their ranges overlap considerably for both, some confusion in assignment of pair number may have occurred. The variation in arm ratios for Pair V is unusually high for some

TABLE XIII

Neophorocera hamata,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1 ♀	1.33	1.85	1.51	1.63	1.53	1.27	5.1	16.1	17.3	18.5	19.4	23.7	84.1
1 ♀	1.27	2.00	1.51	1.45	1.84	1.51	5.0	15.0	17.7	18.7	19.4	24.3	77.9
1 ♀	1.33	1.74	1.31	1.35	1.88	1.38	5.0	16.5	17.3	18.7	19.5	23.0	84.7
2 ♀	1.33	1.89	1.75	1.33	2.12	1.35	4.6	17.0	18.2	18.3	19.7	22.1	93.1
3 ♂	1.25	1.39	1.81	1.61	1.49	1.41	4.8	16.9	18.4	18.4	19.3	22.2	59.6
4 ♀	1.25	1.54	1.71	1.85	1.50	1.33	4.5	17.1	18.0	18.0	19.7	22.7	60.8
4 ♀	1.25	1.68	1.38	1.67	2.35	1.31	4.8	16.8	17.3	18.0	19.2	23.9	60.1
4 ♀	—	1.38	1.56	1.61	1.92	1.50	5.3	15.9	16.9	19.5	20.0	22.5	56.2
5 ♀	1.57	1.50	1.88	1.47	1.90	1.38	4.7	16.6	17.0	18.6	19.8	23.3	63.5
5 ♀	—	2.09	1.58	1.57	1.96	1.34	5.2	16.2	16.7	17.8	19.0	25.1	60.6
5 ♀	2.00	1.79	1.56	1.44	1.83	1.34	5.2	16.7	17.2	18.1	20.3	22.4	60.4
5 ♀	1.54	1.75	1.60	1.32	1.70	1.37	3.9	17.1	17.5	18.4	19.4	23.7	58.4
Average	1.41	1.72	1.60	1.53	1.84	1.37	4.8	16.5	17.5	18.4	19.6	23.2	68.3

unknown reason. The high average for the arm ratios of Pairs II–VI (1.60) clearly places this species in the high arm-ratio group. No secondary constrictions were found.

The *Y*-chromosome was very tiny and indistinct. Nevertheless, it corresponded to 71.6% of the *X*-chromosome in length (corresponding to 3.4% of the TCL) for three cells analyzed in the single male specimen available. The arm ratios averaged 1.10 for two *Y*-chromosomes. The *X*-chromosome stained clearly at metaphase but was not heterochromatic and heteropycnosis was not observed in metabolic nuclei.

A single tetraploid cell was found at metaphase (Plate II, Fig. 12). It is clear in this figure that all four homologues remained together and apparently retained their affinity for each other (confirming previous reports on mitotic pairing in tetraploid cells of Diptera). The results of analysis are those for the last cell of 5 ♀ listed in Table XIII.

Smith (8) has illustrated the somatic complement of this species. Since the kinetochores were very clear and all parts appeared to be in the same focus in his figure, an analysis was attempted. Such an analysis is, of course, likely to be inaccurate but the results did not differ in any important respect from those presented above.

Omotoma fumiferanae (Tot.)

The larval material studied was reared on the spruce budworm. It was possible to analyze somatic chromosome complements from one male larva and three male and two female pupae. All had 12 chromosomes. The longest

TABLE XIV

Omotoma fumiferanae,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)*	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1♂	—	1.50	1.58	1.43	1.21	1.08	11.3	13.7	15.4	16.7	19.6	23.2	65.5
2♂	—	1.43	1.74	1.47	1.21	1.07	10.4	14.5	15.7	15.7	19.5	24.1	71.0
2♂	4.25	1.32	1.64	1.52	1.16	1.14	10.6	14.3	16.1	16.3	20.2	22.5	61.9
2♂	2.67	1.44	2.00	1.41	1.27	1.08	10.8	14.1	14.5	16.5	21.1	23.0	65.6
3♀	3.40	1.36	1.62	1.36	1.19	1.07	11.8	13.8	14.6	15.5	20.5	23.9	57.8
4♀	4.50	1.27	1.67	1.29	1.18	1.09	11.0	14.4	15.5	15.5	20.8	22.8	62.3
4♀	4.50	—	1.41	1.27	—	1.08	10.0	15.5	15.5	16.5	20.4	22.4	69.7
4♀	3.62	1.27	1.94	1.41	1.17	1.10	10.4	15.2	15.2	16.0	18.8	24.5	50.7
5♂	4.22	1.38	1.72	1.38	1.30	1.12	12.0	14.1	15.4	15.7	19.1	23.6	66.6
5♂	3.20	1.29	1.77	1.29	1.37	1.14	11.3	14.4	15.9	16.2	19.3	23.0	58.2
6♂	4.20	1.41	1.96	1.29	1.22	1.03	10.4	14.1	14.7	15.9	20.5	24.4	73.6
6♂	5.25	1.27	1.45	1.36	1.16	1.07	11.2	13.1	15.6	15.6	19.8	24.6	68.3
Average	3.98*	1.36	1.71	1.37	1.22	1.09	10.9	14.2	15.3	16.0	20.0	23.5	64.3

* *Probably secondary ratios.*

complement had a TCL of 73.6 μ and the shortest 50.7 μ, with an average for 12 cells of 64.3 μ. The chromosomes are illustrated in Plate II, Fig. 13, and results of analyses are recorded in Table XIV and Text-fig. 10.

Chromosome Pairs I, V, and VI are distinct from the other pairs in length. There is some overlapping in the values of percentages of TCL for Pairs II and III and for Pairs III and IV. However, the relatively higher arm ratio of Pair III (1.73 as compared with 1.36 for II and 1.37 for IV) greatly facilitates recognition of these pairs. The average of arm ratios for Pairs II–VI is 1.35 and accordingly this species belongs to the low arm-ratio group.

Chromosome I (X) has two distinct constrictions. They divide the chromosome into three portions having an average ratio of 1.00 : 2.40 : 1.58. From metaphase associations of the X and Y, it seems that the kinetochore is at the outer end of the shortest segment, but this is not yet certain. If the secondary constriction closest to the kinetochore is taken as the main secondary constriction, the average secondary arm ratio is 3.98. The longer segment, distal to the main secondary constriction, has two parts separated by a very distinct constriction (a wide, achromatic region), so that the proximal portion is 1.52 times the distal portion in length. In one male (6 in Table XIV) these segments had a reverse order so that the proximal segment was only 0.60 times the distal. This male may belong to a separate subgroup of this species. A subterminal secondary constriction near the distal end of the long arm of Chromosome VI was noticed in two cells only.

The Y-chromosome is nearly 80% as long as the X (corresponding to 8.7% of the TCL) but lacks one distinct secondary constriction, i.e., it has only one constriction. The arm ratio (probably secondary) of the Y was much lower than that of the X (2.25 for seven cells and 3.98 respectively). Although neither of these chromosomes stained differentially at metaphase, there were distinct indications of heteropycnosis (which presumably involved the X-chromosome) in metabolic nuclei.

Phryxe pecosensis (Tns.)

Larvae used in the study of this species were reared on the spruce budworm. The 12 cells analyzed were from three male and two female third instar larvae, and each contained 12 chromosomes. Their complements were small, ranging from 42.4 μ to 54.5 μ with an average of 48.9 μ for total complement length. The results of analysis are presented in Table XV and Text-fig. 11, and the chromosomes are illustrated in Plate II, Figs. 14 and 15.

The X-chromosome (Pair I) is short in this species and much shorter than Pair II. Pair II differs from III by only 0.9%, III from IV by 0.8%, and IV from V by 1.4% of the TCL. Under these circumstances occasional errors in assignment to pair may have occurred. The arm ratios differ only slightly between these pairs and, accordingly, are not very useful supplements in pair assignment. The average of arm ratios for Pairs II–VI, 1.17, places this species in the low arm-ratio category.

TABLE XV

Phryxe pecosensis, 1949,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(X)	II	III	IV	V	VI	I(X)	II	III	IV	V	VI	
1♀	—	1.25	1.27	1.29	1.33	1.10	5.7	15.8	17.2	18.5	20.4	22.4	52.2
2♂	2.33	1.08	1.20	1.07	1.18	1.12	7.7	17.1	17.5	17.8	18.6	21.3	48.1
2♂	—	1.07	1.28	1.38	1.27	1.05	6.6	16.5	16.5	17.9	19.8	22.8	51.8
2♂	—	1.14	1.24	1.13	1.12	1.06	7.4	16.4	17.1	17.7	19.1	22.4	42.4
2♂	—	1.08	1.12	1.33	1.18	1.06	7.7	16.1	17.3	18.1	19.3	21.4	47.8
3♂	2.67	1.07	1.26	1.32	1.25	1.13	6.3	16.7	16.9	18.0	19.9	22.4	54.5
3♂	2.67	1.07	1.18	1.07	1.15	1.08	6.5	16.8	17.1	17.3	19.8	22.5	52.5
3♂	3.00	1.12	1.12	1.28	1.32	1.12	6.1	17.1	17.4	18.6	18.9	21.9	46.7
3♂	2.17	1.08	1.11	1.10	1.13	1.12	5.7	16.0	18.0	18.5	20.0	21.7	49.8
4♀	3.00	1.18	1.15	1.33	1.23	1.23	6.6	15.5	18.5	18.8	19.1	21.9	46.3
4♀	2.33	1.08	1.25	1.11	1.18	1.03	6.9	16.3	17.4	18.0	18.9	22.5	47.4
5♂	—	1.08	1.12	1.25	1.21	1.06	6.0	16.2	17.1	17.4	20.4	22.9	47.4
Average	2.60	1.11	1.19	1.22	1.21	1.10	6.6	16.4	17.3	18.1	19.5	22.2	48.9

A secondary constriction was noticed near the middle of the short arm of Chromosome VI in five cells only. Otherwise secondary constrictions were conspicuously absent.

The acrocentric *Y*-chromosomes are very short (59% of the *X*, corresponding to 4.1% of the TCL for the average of nine) and stain lightly. Their secondary arm ratios averaged 1.13 for four cells where they were obtainable. The acrocentric *X*-chromosomes did not stain conspicuously at metaphase in this species, but the shorter proximal segment stained more lightly than the longer distal segment.

A single tetraploid cell was found in one male. The association of homologues in fours was less obvious than in the tetraploid cell found in *Neophorocera hamata*.

One individual (presumably a female) had a chromosome complement that differed conspicuously in morphology from the others. The analysis of one cell gave the results shown below:

	I	II	III	IV	V	VI
Arm ratio	1.37	1.15	1.37	1.55	1.30	1.04
Percentage of TCL	14.1	15.8	16.5	16.9	16.9	19.9

The TCL was 42.3 μ, the average of arm ratios for II–VI was 1.28, and no secondary constrictions were found. This analysis corresponds sufficiently well to that of *Madremyia saundersii* for it to be a member of that species. Its atypical nature was immediately recognized in a casual observation of its chromosomes even before analysis.

The initial analysis shown above was done in 1949. In 1951 two more larvae of this species were obtained, a male and a female. The results of analyses of the somatic complements in nine cells from these two larvae are shown below:

	(*Y*)	I	II	III	IV	VI	VI
Arm ratio	1.76?	2.42	1.19	1.16	1.26	1.16	1.13
Percentage of TCL	(4.4)	6.5	16.5	17.5	18.2	19.3	22.0

In general these results are remarkably similar to those previously obtained and clearly show that, though variation exists between individual cells, the average results are reproducible with encouraging accuracy. It should also be mentioned that the 70% and 95% ethyl alcohols were omitted in the preparation of slides for the second series of analyses. The modification does not seem to have seriously affected the results.

Spathimeigenia sp.

The third instar larvae of this species were reared on the red-headed pine sawfly. The four males and two females studied all contained 12 chromosomes in each of the 12 cells analyzed. Their somatic complements ranged from 44.0 μ to 71.9 μ in total complement length, with an average of 53.8 μ. The male complement is shown in Plate II, Fig. 16, and the results of analyses in Table XVI and Text-fig. 12.

TABLE XVI

Spathimeigenia SP.,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1♂	3.00	1.17	1.50	1.47	2.20	1.41	5.0	13.9	17.1	18.1	20.8	25.0	57.4
1♂	—	1.25	1.91	1.52	1.96	1.49	6.2	14.9	17.5	18.5	19.2	23.8	55.5
2♂	—	1.40	1.68	1.50	2.00	1.69	5.0	14.4	17.4	19.1	19.3	24.8	51.5
2♂	—	1.57	1.68	1.47	2.20	1.51	5.7	14.1	16.7	18.4	19.8	25.2	60.4
2♂	3.04	1.50	1.72	1.55	1.85	1.43	7.1	14.2	17.2	17.8	19.7	24.0	44.0
2♂	—	1.33	1.56	1.41	2.10	1.49	4.9	14.5	17.3	18.9	19.5	24.9	52.0
2♂	—	1.32	1.80	1.46	1.91	1.59	6.1	13.6	17.3	18.2	19.7	25.1	49.3
3♂	—	1.28	1.50	1.41	1.85	1.35	6.3	13.5	17.6	18.4	19.2	25.0	71.9
4♀	—	1.19	1.57	1.83	2.25	1.60	7.8	13.9	17.4	19.1	19.1	22.7	51.4
5♂	—	1.27	1.77	1.57	2.32	1.42	6.1	13.8	16.6	19.2	19.7	24.6	55.7
5♂	2.67	1.42	1.60	1.33	1.87	1.46	6.9	14.3	16.0	19.1	19.4	24.3	49.8
6♀	2.50	1.19	1.80	1.44	2.22	1.41	5.4	15.1	18.1	19.6	19.6	22.3	47.3
Average	2.80	1.32	1.67	1.50	2.06	1.49	6.0	14.2	17.2	18.7	19.6	24.3	53.8

The chromosome pairs of this species are rather distinct both in length, expressed as percentage of TCL, and in arm ratio. With such differences there is very slight chance of confusion in assignment to pair. The high average (1.61) arm ratio for Pairs II–VI places this species in the high arm-ratio group.

Secondary constrictions were found in all of the chromosomes. They were more frequently seen in the longer complements. Chromosome II has a near-medial constriction in the short arm and sometimes a subterminal one in the same arm. The constriction in the shorter arm of III is near the middle in two cases and near the distal end in four. In half the cells analyzed a constriction was seen near the distal end of the long arm of Chromosome IV. Similarly, constrictions were found near the distal end of the longer arm of Chromosome V. Also, secondary constrictions were observed about the middle of the short arm of VI, occasionally near the distal end of this arm, and in about half the cells near the distal end of the long arm. It is possible that such constrictions occur at weaker points in these chromosomes when pressure is applied during squashing. On the other hand, their conspicuousness may be influenced by differences in degree of contraction in different cells. Whatever the reason, the inability to locate them regularly reduces their value in identifying chromosome pairs.

The *Y*-chromosome is about 74% of the *X* in length, corresponds to 4.4% of the TCL, and is often very inconspicuous and hard to find since it stains very lightly. Its arm ratio averaged 1.17 for five chromosomes. Heteropycnosis is not conspicuous in metabolic nuclei and the *X*-chromosome does not appear to be differentially stained at metaphase.

One larva, not included above, had a complement that differed in certain respects. The analyses of four figures from this larva are summarized below:

	I	II	III	IV	V	VI
Arm ratio	2.07	1.42	1.90	1.52	2.30	1.37
Percentage of TCL	3.6	17.3	17.6	18.4	18.5	24.7

The differences between these results and those for other larvae of this species are apparent. The unusually large size of the complements analyzed may account for this peculiar result but possibly this individual belongs to a different subgroup of this species.

Winthemia datanae (Tns.)

Twelve third instar larvae of this species were obtained but cells suitable for analysis were found in only four. From the presence of two extremely large heterochromatic chromosomes (which are presumably the *X*-chromosomes) all 12 individuals were assumed to be females. Each of the 12 cells analyzed

had a somatic complement of 12 chromosomes. The complements ranged from 47.4 μ to 72.7 μ with 54.0 μ as the average and thus were smaller than those of *Winthemia occidentis*. The somatic complement is illustrated in Plate II, Fig. 17, and analyses are presented in Table XVII and Text-fig. 13.

TABLE XVII

Winthemia datanae,—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(X)*	II	III	IV	V	VI	I(X)	II	III	IV	V	VI	
1♀?	1.19	1.38	1.60	1.10	1.40	1.43	31.0	12.2	12.5	13.3	15.1	15.9	49.1
2♀?	1.09	1.16	1.39	1.09	1.32	1.61	29.5	13.2	13.8	14.1	14.4	15.0	48.4
2♀	1.15	1.35	1.62	1.20	1.37	1.81	30.4	13.0	13.6	14.2	14.4	14.4	48.3
2♀	1.10	1.18	1.45	1.20	1.37	1.81	31.9	11.7	13.7	13.7	14.1	14.9	49.7
2♀	1.20	1.39	1.53	1.15	1.40	1.55	29.4	13.4	13.4	13.4	14.8	15.7	50.0
3♀?	1.14	1.21	1.19	1.29	1.40	1.41	33.2	11.9	13.0	13.5	13.5	14.8	54.8
3♀	1.10	1.25	1.38	1.13	1.36	1.34	33.9	11.3	12.5	12.7	12.9	16.6	61.6
3♀	1.13	1.29	1.35	1.21	1.26	1.35	30.6	12.9	13.2	13.8	14.1	15.3	47.4
3♀	1.07	1.22	1.26	1.32	1.78	1.48	33.3	11.4	12.2	14.3	14.1	14.6	54.7
3♀	1.13	1.14	1.30	1.45	1.41	1.52	30.9	12.5	12.8	13.5	14.5	15.8	55.8
3♀	1.09	1.19	1.30	1.17	1.60	1.19	29.8	13.0	13.0	14.0	14.5	15.8	54.9
4♀?	1.21	1.21	1.42	1.06	1.27	1.23	27.6	13.3	13.1	14.5	15.4	16.0	72.7
Average	1.13*	1.25	1.40	1.20	1.42	1.47	31.0	12.5	13.1	13.8	14.3	15.4	54.0

* *True arm ratios, i.e., X is metacentric.*

The very large metacentric Pair I is assumed to be the *X*-pair because these chromosomes are heterochromatic, resembling the *X*-pair of *W. occidentis*. Pair I is much larger here (31.0% TCL) than in *W. occidentis*, in which it averages 16.2% of the TCL. As a result, the percentage of TCL differences between the other pairs are reduced in *W. datanae*. The arm ratios and secondary constrictions are helpful in the assignment of chromosomes to pair but analysis of some complements was still very difficult and some confusion of pairs may have persisted.

The primary constriction in Chromosome I is clearly submedial in this species (Plate II, Fig. 17). Pairs III, V, and VI commonly have arm ratios higher than those of Pairs II and IV. However, none of these pairs differs very much from the others. The average arm ratio, 1.34, for Pairs II–VI places this species in the low arm-ratio group. A subterminal secondary constriction was found near the distal end of the long arm of Chromosome I and another near the proximal end of its short arm. One of these is usually detectable and both in some cells. Chromosome VI usually has a distinct secondary constriction in the medial or submedial position of its longer arm.

No *Y*-chromosome has been identified in any individual of which preparations were available. The *X*(?)-chromosome is strongly heterochromatic at

metaphase and heteropycnotic in metabolic nuclei. The metabolic nuclei have very large nucleoli and it is tempting to assume that much of their substance is transferred to the very large heterochromatic Chromosome I during mitosis.

Winthemia occidentis Rnh.

The larvae of this species were parasites in the oak looper, *Lambdina fiscellaria somniara*, collected in British Columbia. Only limited material was available but it was possible to analyze 12 cells from two female and three male third instar larvae. In all cells 12 chromosomes were found. These complements ranged from 50.0 μ to 79.4 μ in total complement length, with 64.0 μ as the average. The complements of male and female are illustrated in Plate II, Figs. 18, 19, and 20. Table XVIII and Text-fig. 14 show the results of analysis.

TABLE XVIII

Winthemia occidentis.—ANALYSES OF 12 SOMATIC COMPLEMENTS

Larva number	Arm ratios for pairs						Percentages of TCL for pairs						TCL in μ
	I(*X*)*	II	III	IV	V	VI	I(*X*)	II	III	IV	V	VI	
1♀	9.05	1.12	1.04	1.32	1.36	1.42	15.2	14.0	14.9	17.7	18.9	19.4	58.1
2♀	6.58	1.00	1.15	1.37	1.75	1.29	13.8	15.0	15.5	17.1	17.5	21.1	56.8
3♂	6.00	1.45	1.00	1.38	1.36	1.37	15.1	13.8	15.6	17.2	18.8	19.5	54.7
4♂	6.50	1.25	1.09	1.08	1.17	1.41	17.7	14.0	14.2	15.4	19.1	19.7	50.0
5♂	5.83	1.10	1.12	1.05	1.38	1.36	16.9	13.9	15.3	16.1	18.5	19.3	70.7
5♂	6.20	1.07	1.13	1.20	1.38	1.37	15.4	13.7	15.0	17.2	19.1	19.5	68.5
5♂	8.75	1.15	1.18	1.41	1.33	1.34	17.5	13.6	14.2	17.7	18.2	18.9	65.0
5♂	9.50	1.22	1.41	1.40	1.38	1.34	15.4	13.5	15.4	18.0	18.6	19.2	79.4
5♂	7.75	1.04	1.09	1.29	1.55	1.33	15.9	13.0	15.6	18.0	18.2	19.3	64.8
5♂	7.40	1.07	1.13	1.14	1.33	1.30	18.1	13.5	14.7	16.6	18.5	18.7	67.8
5♂	8.00	1.10	1.00	1.38	1.47	1.26	15.5	14.5	15.5	17.8	17.8	18.9	68.9
5♂	8.75	1.08	1.00	1.22	1.39	1.24	17.9	12.0	13.4	18.8	18.5	19.4	63.8
Average	7.53	1.14	1.11	1.27	1.38	1.34	16.2	13.7	15.0	17.3	18.5	19.4	64.0

* *Secondary arm ratios.*

The acrocentric *X*-chromosome (Pair I) is very large in this species. Pair I usually exceeds Pairs II and III and exceeds Pair IV in 25% of the cells. There was usually no great difficulty in distinguishing other pairs on the basis of differences in length, but arm ratios were referred to for some assignments of pair numbers. The arm ratios for Pairs II–VI average 1.25 which places this species in the low arm-ratio category.

At first one is inclined to consider the prominent submedial secondary constriction in the *X* as the kinetochore. However, the anaphase figure (Plate II, Fig. 19) clearly shows that the primary constriction is at or very near one end and in metaphase figures of cells of females it is clear that attraction between

the two *X*-chromosomes (Pair I) is strong only near one end. The long segment with reference to the proximal subterminal secondary constriction (used as the main secondary constriction) is about six to nine times as long as the short segment. The exact position of the submedial constriction in the long segment varies somewhat but the proximal part (i.e., next to the subterminal secondary constriction) averaged 79.5% of the distal part. In two cases this distal part was subdivided into two by an achromatic band.

The shorter arm of Pair II has a nearly medial secondary constriction observable in nearly all cells. Subterminal secondary constrictions were found on the short arms of Pairs V and VI.

Ten *Y*-chromosomes averaged 36.5% of the *X*, corresponding to 6.0% of the TCL, and eight of them had secondary arm ratios which averaged 2.88. This *Y* is larger than the *X*-chromosome in some other species of Tachinidae (e.g., *Neophorocera hamata*). The distal part of the long segment of the *X* is noticeably heterochromatic but the *Y* and other parts of the *X* were not noticeably so at metaphase. Probably, at least a part of the *X*-chromosome is heteropycnotic since long, dense chromatic bodies are observable in metabolic nuclei.

Comparison of Somatic Complements of Tachinid Species

The data from the analyses of the 16 species of Tachinidae studied to date have been collected for direct comparison in Table XIX. Most of these complements are represented in Text-figs. 3 to 14.

It is clear that five of the species listed have a much higher average arm ratio for Pairs II–VI. The average arm-ratio figures are probably very dependable since each represents the average of values for 120 separate chromosomes when 12 cells were analyzed. On this basis *Neophorocera hamata*, *Spathimeigenia* sp., *Aplomya mitis*, *Aplomya caesar*, and *Ceracia dentata* constitute a separate group all of which have high average arm ratios (1.53–1.72). In the remaining group these average ratios range from 1.17 to 1.35.

The members of the high arm-ratio group may be separated further on the basis of size and arm ratios of the *X*-chromosomes (Pair I). The *Aplomya* species have distinctly longer *X*-chromosomes (9.1% and 11.3%) than those of *Neophorocera hamata* (4.8%), *Spathimeigenia* sp. (6.0%), or *Ceracia dentata* (6.2%). The two *Aplomya* species differ appreciably from each other in the length of the *X*-chromosomes as indicated above, and these chromosomes also differ in morphology as previously mentioned. The arm ratios for chromosome pairs I, II, and V of *Neophorocera hamata* and *Spathimeigenia* sp. differ strikingly and should serve as a satisfactory basis for their separation. *Ceracia dentata* has a large *Y* (5.6%); and other distinctive chromosomal characters by which it can be distinguished from the last two species mentioned.

In the low arm-ratio group several subgroups are distinguishable on the basis of size of the *X*-chromosomes:

Subgroup I, species having small *X*-chromosomes ranging from 5.5% to 7.5% of the TCL. This subgroup includes *Drino bohemica*, *Phryxe pecosensis*, *Eumea westermanni*, *Mericia ampelus*, and *Ceromasia auricaudata*.

TABLE XIX

COMPARISON OF SOMATIC CHROMOSOME COMPLEMENTS IN THE TACHINIDAE

Species	Y-chromosome		Chromosome pair												Av. arm ratio II–VI	Av. TCL, μ	No. of cells analyzed
			I		II		III		IV		V		VI				
	% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio			
Aplomya caesar	3.5	1.00	11.3	1.68	14.6	1.54	15.6	1.63	16.6	1.71	18.2	2.14	23.6	1.50	1.70	57.7	12
Aplomya mitis	4.0	1.25	9.1	1.51	14.3	1.40	15.9	1.50	17.4	1.94	19.3	2.25	24.0	1.51	1.72	45.5	12
Ceracia dentata	5.6	1.25	6.2	2.61	16.8	1.09	17.1	1.67	17.9	1.23	18.7	2.38	23.4	1.27	1.53	52.7	4
Ceromasia auricaudata	4.4	1.22*	6.0	1.67*	15.7	1.20	17.0	1.22	17.8	1.32	18.5	1.13	25.1	1.27	1.23	54.8	12
Drino bohemica	3.8	1.21	7.4	2.09	14.7	1.14	16.5	1.13	17.5	1.32	19.1	1.16	24.8	1.39	1.22	63.7	27
Eumea westermanni	—	—	5.9	2.17	16.3	1.95	17.4	1.03	19.1	1.13	20.1	1.13	21.3	1.12	1.28	50.8	1
Lydella grisescens	18.4	1.24†	19.8	1.25†	14.2	1.20	14.8	1.12	15.9	1.17	16.5	1.23	19.0	1.35	1.22	69.8	4
Madremyia saundersii	4.3	1.00?	12.9	1.43	14.9	1.22	16.3	1.26	17.0	1.26	18.4	1.24	20.5	1.11	1.22	52.8	12
Mericia ampelus	3.6	—	7.0	1.12	15.5	1.84	16.7	1.36	18.6	1.30	19.7	1.20	22.5	1.05	1.35	42.7	2
Nemorilla pyste	—	—	13.0	1.18	15.3	1.34	15.7	1.07	16.6	1.37	18.4	1.19	21.0	1.10	1.21	55.4	3
Neophorocera hamata	3.4	1.10	4.8	1.41	16.5	1.72	17.5	1.60	18.4	1.53	19.6	1.84	23.2	1.37	1.60	68.3	12
Omotoma fumiferanae	8.7	2.25*	10.9	3.97*	14.2	1.36	15.3	1.71	16.0	1.37	20.0	1.22	23.5	1.09	1.35	64.3	12
Phryxe pecosensis	4.1	1.13*	6.6	2.60*	16.4	1.11	17.3	1.19	18.1	1.22	19.5	1.21	22.2	1.10	1.17	48.9	12
Spathimeigenia sp.	4.4	1.17	6.0	2.80	14.2	1.32	17.2	1.67	18.7	1.50	19.6	2.06	24.3	1.49	1.61	53.8	12
Winthemia datanae	—	—	31.0	1.13†	12.5	1.25	13.1	1.40	13.8	1.20	14.3	1.42	15.4	1.47	1.34	54.0	12
Winthemia occidentis	6.0	2.88*	16.2	7.53*	13.7	1.14	15.0	1.11	17.3	1.27	18.5	1.38	19.4	1.34	1.25	64.0	12

* *Secondary arm ratios.*

† *Primary arm ratios.*

All other sex chromosomes are presumably acrocentric and are assumed to have secondary arm ratios.

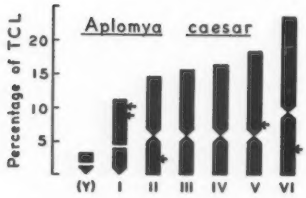


Figure 3

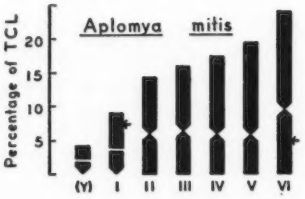


Figure 4

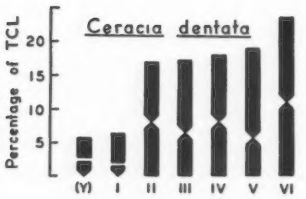


Figure 5

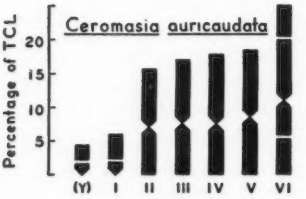


Figure 6

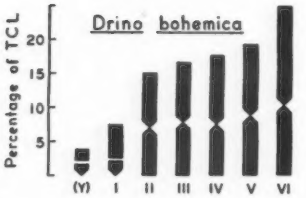


Figure 7

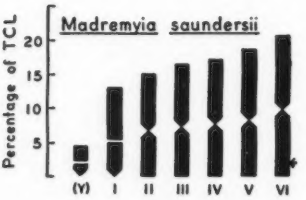


Figure 8

TEXT-FIGS. 3–8. Idiograms of the somatic complements of species of Tachinidae. See text for explanation.

These species can be distinguished from each other, with one exception, on the basis of arm ratios of their *X*-chromosomes. In the exceptional case, *Drino bohemica* differs from *Eumea westermanni* (if the single cell analyzed is typical) in the arm ratios for Pairs II and VI.

Subgroup II, species having medium-sized *X*-chromosomes ranging from 10.5% to 13.0% of the TCL. The three species included in this subgroup are *Nemorilla pyste*, *Madremyia saundersii*, and *Omotoma fumiferanae*. The first has arm ratios of 1.18 and 1.07 for Pairs II and III respectively, whereas *Madremyia saundersii* has 1.43 and 1.26. *Omotoma fumiferanae* has two distinct constrictions in the chromosomes of Pair I and a large *Y*-chromosome that considerably exceeds that of *M. saundersii* and may exceed that of *N. pyste*, which has not yet been seen.

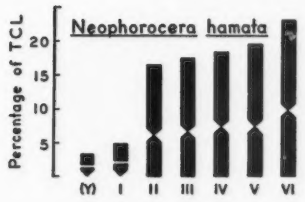


Figure 9

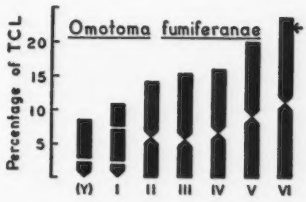


Figure 10

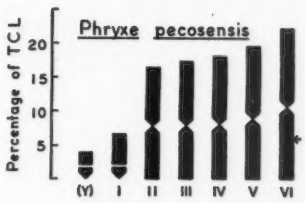


Figure 11

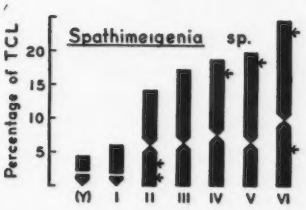


Figure 12

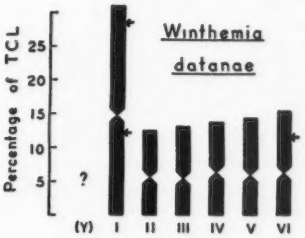


Figure 13

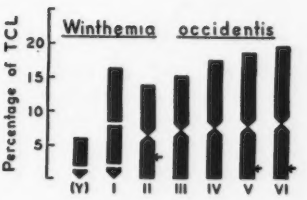


Figure 14

TEXT-FIGS. 9–14. Idiograms of the somatic complements of species of Tachinidae. See text for explanation.

Subgroup III has only one species, *Winthemia occidentis*, which has a large (16.2% of TCL) acrocentric and heterochromatic *X*-chromosome and a relatively large *Y*-chromosome (6.0% of the TCL).

Subgroup IV has only one species, *Lydella grisescens*, which has a large (19.8% of the TCL) metacentric and heterochromatic *X*-chromosome. The *Y* is also large (18.4% of the TCL), metacentric, and heterochromatic.

Subgroup V has only one species, *Winthemia datanae*, which has a very large (31.0% of TCL) metacentric and heterochromatic *X*-chromosome. The *Y*-chromosome of this species has not yet been identified. These subgroups are so distinct that they do not overlap in the size ranges of individual pairs of *X*-chromosomes (as % of TCL).

A general system of separation of the species of Tachinidae as outlined is shown below.

CHROMOSOMAL SEPARATION OF SPECIES OF TACHINIDAE

Species with 12 somatic chromosomes
- Average arm ratio of Pairs II–VI about 1.50–1.75
  - *X*-chromosomes 4.5% to 6.5% of TCL
    - *X* arm ratio 1.4, II arm ratio 1.7 ..... *Neophorocera hamata*
    - *X* arm ratio 2.6, II arm ratio 1.3 ..... *Ceracia dentata*
    - *X* arm ratio 2.8, II arm ratio 1.1 ..... *Spathimeigenia* sp.
  - *X*-chromosomes 9.0% to 11.5% of TCL
    - *X*-chromosome about 9.1% of TCL ..... *Aplomya mitis*
    - *X*-chromosome about 11.3% of TCL ..... *Aplomya caesar*
- Average arm ratio of Pairs II–VI about 1.15–1.35
  - *X*-chromosomes small, 5.5% to 7.5% of TCL
    - *X* arm ratio about 1.10 ..... *Mericia ampelus*
    - *X* arm ratio about 1.70 ..... *Ceromasia auricaudata*
    - *X* arm ratio about 2.10
      - Pair II arm ratio about 1.15 ..... *Drino bohemica*
      - Pair II arm ratio about 1.95(?) ..... *Eumea westermanni*
    - *X* arm ratio about 2.60 ..... *Phryxe pecosensis*
  - *X*-chromosomes medium-sized, 10.5% to 13.0% of TCL
    - *X* arm ratio 1.2, Pair III ratio 1.1 ..... *Nemorilla pyste*
    - *X* arm ratio 1.4, Pair III ratio 1.25 ..... *Madremyia saundersii*
    - *X* two constrictions and *Y* large (8.7%) ..... *Omotoma fumiferanae*
  - *X*-chromosome about 16% of TCL, acrocentric ..... *Winthemia occidentis*
  - *X*-chromosome about 20% of TCL, metacentric ..... *Lydella grisescens*
  - *X*-chromosome about 31% of TCL, metacentric ..... *Winthemia datanae*

Separation of species within most of the subgroups is definite. The *X*-chromosome values for arm ratios in *Neophorocera hamata* do not overlap those of the *Spathimeigenia* species; also, the corresponding values for percentage of the TCL overlap very slightly although the averages differ by only 1.2% of the TCL. The ranges in percentage of TCL for *X*-chromosomes of the two *Aplomya* species (Tables IV and V) overlap slightly but there are also differences in chromosome morphology between them. In the low arm-ratio group three species, *Mericia ampelus*, *Exorista westermanni*, and *Nemorilla pyste*, must be considered as being only tentatively assigned to their present positions in the table since so few cells were analyzed for each. Further work on *Nemorilla pyste* is needed to confirm the differences indicated between this species and *Madremyia saundersii*. The remaining species in the low arm-ratio group are distinct cytologically.

It should be kept in mind that the detailed descriptions of the somatic complements of these species, as previously outlined, afford abundant supplementary data of value in checking the above specific system of identification. The general relations of different pairs as percentages of the total should be useful; for example, compare the *Omotoma* species with those in *Winthemia*. The secondary constrictions may be very valuable in certain cases (Pair VI of *Ceromasia auricaudata*, Text-fig. 6 and Plate I, Fig. 6). Differences in heteropycnosis, though not stressed above, should be useful also. Corresponding parts of chromosomes can be compared directly in the figures. All of these

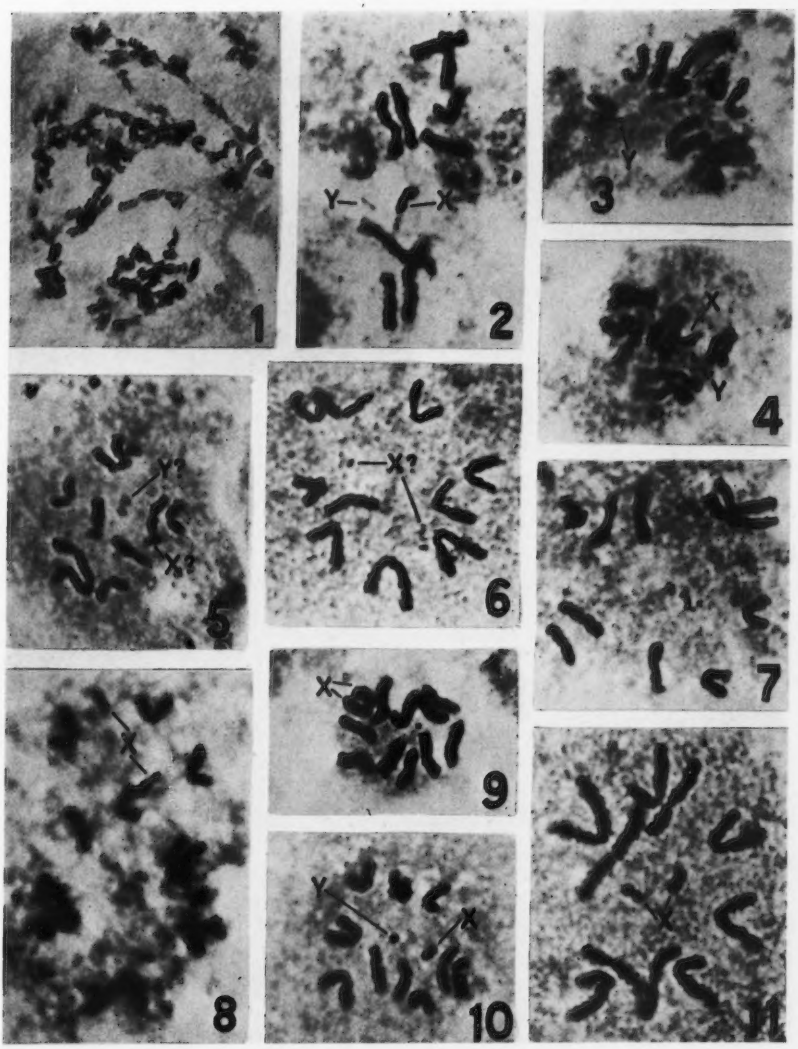
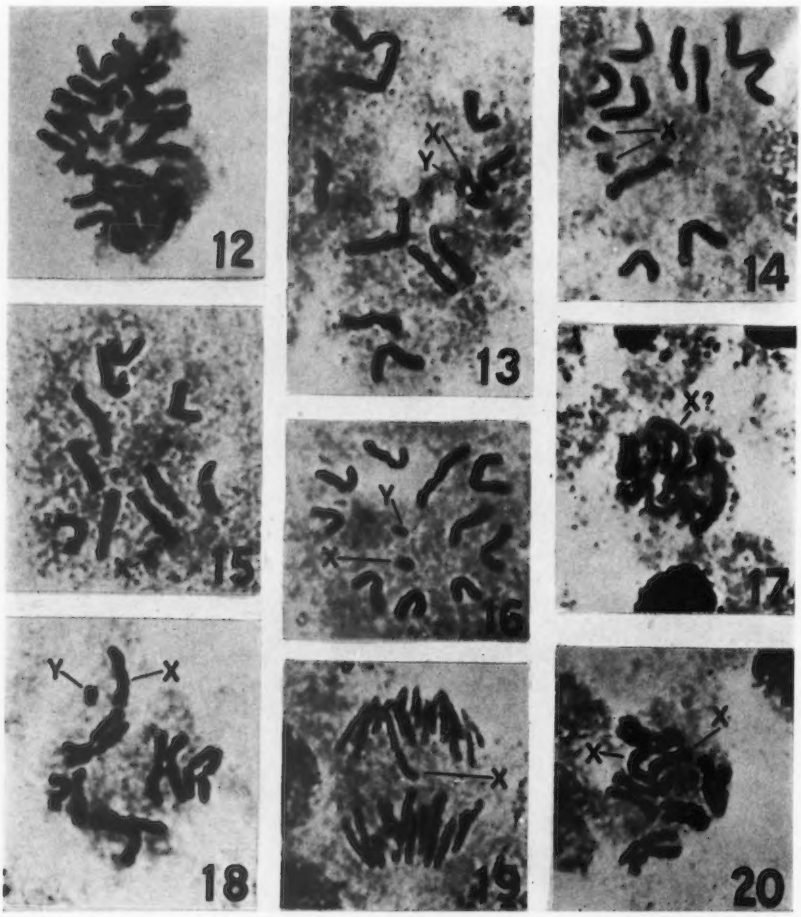


FIG. 1. Salivary chromosomes of *Drino bohemica*. ×290.

FIGS. 2–11. Somatic chromosome complements at metaphase in brain cells. ×1800.

FIG. 2. *Aplomya caesar* male from the European corn borer. FIG. 3. *Aplomya caesar* male from the spruce budworm. FIG. 4. *Aplomya mitis* male; note remains of nucleolus, bottom center. FIG. 5. *Ceracia dentata* male. FIG. 6. *Ceromasia auricaudata* female (?); *X*'s are tiny and partly light-staining. FIG. 7. *Ceromasia auricaudata* male (?) with a supernumerary. FIG. 8. *Drino bohemica* female, remains of nucleolus adjacent to large chromosome at lower left. FIG. 9. *Madremyia saundersii* female. FIG. 10. *Mericia ampelus* male. FIG. 11. *Neophorocera hamata* female.



FIGS. 12–20. Somatic chromosome complements at metaphase (except Fig. 19) in brain cells. ×1800.

FIG. 12. *Neophorocera hamata* female, tetraploid cell. FIG. 13. *Omotoma fumiferanae* male. FIG. 14. *Phryxe pecosensis* female. FIG. 15. *Phryxe pecosensis* male, *Y*-chromosome not clear. FIG. 16. *Spathimeigenia* sp. male. FIG. 17. *Winthemia datanae*, sex unknown, *X?* may be *X* or *Y*. FIG. 18. *Winthemia occidentis* male. FIG. 19. *Winthemia occidentis* male, anaphase; note long acrocentric *X* in center. FIG. 20. *Winthemia occidentis* female.

bases of comparison applied simultaneously in the direct comparison of the complements of two similar species should reveal significant differences wherever they exist.

The only record of the chromosomes of the Tachinidae found to date is the single photomicrograph by Smith (8) of a somatic complement of *Neophorocera hamata*. As previously mentioned, analysis of the chromosomes in his figure gives results comparable to ours. Perhaps one should also mention *Bessa selecta*?, which Smith (9) has figured, since Curran (1) places the genus *Bessa* in the Tachinidae separated from most of the Sarcophagidae, which he puts in the Metopiidae. The following analysis has been made from Smith's photograph of this species:

	I	II	III	IV	V	VI
Arm ratio	3.00*	2.05	1.77	1.29	1.62	1.44
Percentage of TCL	4.5	16.6	17.4	17.7	19.3	24.6

* *Probably a secondary ratio.*

The average arm ratio for Pairs II–VI was 1.63. Thus, this species fits into the Tachinidae in the first section of the high arm-ratio group. A casual inspection indicated that it could be distinguished from the other species in that section by the arm ratios of Pairs I, II, and IV.

General Discussion and Conclusions

Twelve somatic chromosomes have been recorded for all species of Calyptratae for which reports have been found in the literature. These reports include only 25 species (the reports for six of which are doubtful) from 15 genera. The present report adds 15 species and greatly extends available information concerning a species previously only figured. These species all have 12 somatic chromosomes with only rare individual specimens having one extra chromosome. Thus for the Calyptratae there is a new total of 40 species, each having 12 somatic chromosomes.

On the other hand, variations in chromosome morphology are common in the Tachinidae. For the *X*-chromosomes these variations include great differences in size (from 4.8% to 31.0% of TCL), in position of the kinetochore, in the positions of secondary constrictions, and in heteropycnosis. Both acrocentric and metacentric *X*-chromosomes were found. The *Y*-chromosomes are much more uniform in size and morphology but they do differ appreciably in the *Omotoma–Winthemia* group. Fortunately, variations in relative size and in positions of the kinetochores also exist in the autosomes of these species.

It may be considered that detailed analysis is unnecessary in many cases. It is true that certain of the above species can be distinguished from others

with only casual observation of the chromosomes. For example, the *X*-chromosome of *Winthemia datanae* is strikingly different from that of *Winthemia occidentis*, and indeed from that of any of the species studied. Accordingly, it must be concluded that the analysis is by no means essential for the separation of many pairs of species. However, it is anticipated that in many cases other species will be found with complements closely resembling those described. Hence details of analysis are presented so that later analyses may be compared directly with those given here, by anyone interested in making such analyses for any species.

The writers are not at all convinced that significant differences exist between the chromosome complements of all species in this family. The existence of the differences outlined above does suggest, however, that the species of this family can be divided on the basis of these differences into well-defined categories of value to the systematist in organizing the family and in identifying larval specimens. In Table I six of the species studied were parasites on the budworm, *C. fumiferana*. These species are *A. caesar*, *C. auricaudata*, *M. saundersii*, *N. pyste*, *O. fumiferanae*, and *P. pecosensis*. When suitable mitotic figures are available the writers are convinced that these species are separable on the basis of chromosome analysis. The host specificity, plus morphological differences of larvae or pupae, plus chromosome analysis should constitute a satisfactory basis for rather precise identification of such parasitic species.

It should be stated that a definite restriction applies to the use of these chromosome analyses in identification of species. Only a few of the species of the Tachinidae have yet been analyzed for chromosome differences. Until more species have been analyzed in this way there must always be a risk of confusing a known with an unknown species. However, within the limits of species studied to date, these chromosome analyses offer a direct method of identifying certain species in the larval stage and in other cases contribute valuable supplementary and confirming data on the identification of specimens.

Analyses of complements of species by the system used here make possible the detection of minor differences between complements of groups within a species (or between separate species grouped together as one species). For example, the probability that somatic complements of *A. caesar* from larvae on the European corn borer differ from those from larvae on the spruce budworm has been mentioned above. Where complements are the same, nothing can be deduced regarding the relationship of such groups within a species. On the other hand, consistent differences in the complements of such groups should indicate the presence of an isolation barrier, though of unknown extent, between them even when morphological or physiological differences have not been previously demonstrated. The detection of such variation within a species population is of obvious theoretical and practical importance.

The results of these analyses should be of some use to systematists who have encountered considerable difficulty in classifying Diptera and should contribute to a better understanding of phylogenetic relationships. For example, the high arm-ratio group consists of species in the genera *Neophorocera*, *Spathimeigenia*,

Aplomya, *Ceracia*, and *Bessa*. Surely we are justified in asking the systematist whether any important morphological common denominator exists in these genera. It may be that such cytological differences are more nearly fundamental than some of the morphological characters in current use.

Chromosome analysis may profitably be made on exotic dipterous parasites before their release in Canada. Even if only certain species can be positively identified in this way, the information would be of decided value in following the spread of introduced species and in assessing their effectiveness as parasites under Canadian conditions.

Acknowledgments

Many persons have contributed to this study. Dr. H. C. Coppel, Mr. R. W. Smith, and Mr. Geo. Wishart, Dominion Parasite Laboratory, Belleville, provided insect larvae that they had collected or reared. Mrs. D. Trasler, Mr. I. Mauer, and Mr. H. Bruneau, made many of the microscope preparations. Mrs. B. C. Boyes helped in making analyses and preparing the manuscript. To these, and to others who contributed directly or indirectly, we extend our sincere thanks.

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